Forty-second Annual
Postgraduate Program

October 17, 2009
Atlanta, GA

Preservation of Ovarian Function

Course 5

Developed in
Cooperation with the
Fertility Preservation
Special Interest Group

Sponsored by the
American Society for
Reproductive Medicine

New Procedure to Obtain CME Credits

Dear Postgraduate Course Participant:

The Accreditation Council for Continuing Medical Education now requires that ASRM document learning for participants in CME programs. Thus, the procedure for claiming CME credits has changed. We ask your cooperation in following the steps below to ensure that your credits are provided correctly to you.

1. Within 3 days after the Annual Meeting you will be sent an email asking you to complete an online evaluation of this postgraduate course. A personalized Web link to the evaluation will be provided in your email. Please do not share this unique link.

2. In late November you will be sent a second email with a personalized Web link asking you to complete the post-test on the content of the course. This test is identical to the pre-test and will enable ASRM to assess the effectiveness of this postgraduate course as a learning activity. For your convenience, the test questions are printed in the course syllabus.

After both steps have been completed, you will be able to claim your CME credits and/or ACOG Cognates and receive a printable CME certificate. Please note that you must provide your 10-digit ACOG Membership Number to have your ACOG Cognates reported to ACOG.

Results of both the course evaluation and the post-test are anonymous.

Both steps must be followed completely by **December 31, 2009** in order to receive CME credits. A maximum of 6.5 CME credits can be claimed for the postgraduate course. Please be aware that some email systems flag emails with Web links as junk mail, and you may need to check your junk-email folder for your notifications.

Please DO NOT forward the links. In case of difficulty please email *pfenton@asrm.org*

********Deadline for receiving CME credits = December 31, 2009********

Continuing Medical Education

Continuing medical education is a lifelong learning modality to enable physicians to remain current with medical advances. The goal of ASRM is to sponsor educational activities that provide learners with the tools needed to practice the best medicine and provide the best, most current care to patients.

As an accredited CME provider, ASRM adheres to the Essentials and policies of the Accreditation Council for Continuing Medical Education (ACCME). CME activities now must first, address specific, documented, clinically important gaps in physician competence or performance; second, be documented to be effective at increasing physician skill or performance; and third, conform to the ACCME Standards for Commercial Support.

AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE

Developed in Cooperation with the

FERTILITY PRESERVATION SPECIAL INTEREST GROUP
ANNUAL MEETING POSTGRADUATE COURSE
ATLANTA, GA
OCTOBER 17, 2009

"PRESERVATION OF OVARIAN FUNCTION"

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All speakers at the 2009 ASRM Annual Meeting and Postgraduate Courses were required to complete a disclosure form. These disclosures were reviewed and potential conflicts of interest resolved by the Subcommittee on Standards of Commercial Support of the Continuing Medical Education Committee. The faculty has revealed the following information as potential conflicts of interest:

Kutluk H. Oktay, M.D.: Nothing to disclose

Ali Eroglu, D.V.M., Ph.D.: Gamete Technology Inc.: Stockholder

Jonathan L. Tilly, Ph.D.: Interest in the intellectual property associated with a patent describing the use of S1P as a therapeutic agent for the prevention of gonadal failure and the preservation of fertility (U.S. Patent Number 7,195,775)

Lynn M. Westphal, M.D.: EMD Serono, Schering Plough, Ferring: Advisory board

This activity may include discussion of off-label or otherwise non-FDA approved uses of drugs or devices.

Accreditation statement:
The American Society for Reproductive Medicine is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

Designation statement:
The American Society for Reproductive Medicine designates this educational activity for a maximum of 6.5 *AMA PRA Category 1 Credits*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

American College of Obstetricians and Gynecologists (ACOG)
The American College of Obstetricians and Gynecologists has assigned 6.5 cognate credits to this activity.

American Board of Bioanalysis (ABB)
The American Society for Reproductive Medicine has been approved to provide Professional Enrichment Education Renewal (PEER) credit through the American Board of Bioanalysis. PEER credit forms for eligible courses are located in the front of this syllabus.

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Please turn off/mute cell phones and pagers during the postgraduate course and all Annual Meeting sessions.

Thank you.

PRESERVATION OF OVARIAN FUNCTION

NEEDS ASSESSMENT AND COURSE DESCRIPTION

Approximately 8% of the more than 662,000 women diagnosed with cancer in the U.S. in 2005 were under the age of 40. While the incidence of cancer in women has increased, the death rate from those cancers has decreased. As more young women survive cancer, the impact of fertility-related complications on their quality of life has begun to gain more attention. The President's Cancer Panel encouraged further research in this area, as well as education and training of physicians and other health professionals. Furthermore, NICHD, in its recent mission statement, included fertility preservation as one of its priorities.

Advances in gonadal, gamete and stem cell biology have revealed theoretical new opportunities for preservation and regeneration of reproductive capacity. While translation from theory to practice requires a sound knowledge of the current concepts of the origin, structure, function and pathophysiology of the ovary, few clinicians have the advanced knowledge of this rapidly changing field of reproductive biology to be able to discriminate between evidence-based and unproven, anecdotal approaches to fertility preservation.

This course will provide reproductive biologists, reproductive endocrinologists, reproductive surgeons and oncologists with the most current information about ovarian biology, stem cells, and mechanisms of gonad failure as they pertain to fertility preservation. The faculty will review the current biological basis for and concerns about a spectrum of methods to preserve fertility. Participants will formulate potential evidence-based approaches to fertility preservation.

ACGME COMPETENCY

Patient Care
Medical Knowledge

LEARNING OBJECTIVES

At the conclusion of this course, participants should be able to:

1. Counsel patients regarding the risk of gonadal failure after cancer treatments.
2. Describe the principles of cryobiology and the main techniques for oocyte and ovarian tissue freezing.
3. Formulate mechanistic, evidence-based approaches to fertility preservation.

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"PRESERVATION OF OVARIAN FUNCTION"
Kutluk H. Oktay, M.D., Chair

Saturday, October 17, 2009

08:15 – 08:30	Course Introduction and Orientation **Kutluk H. Oktay, M.D.**
08:30 – 09:05	Counseling Patients About Reproductive Potential After Cancer Treatment **Lynn M. Westphal, M.D.**
09:05 – 09:15	Questions and Answers
09:15 – 09:50	Practical Cryobiology **Ali Eroglu, D.V.M., Ph.D.**
09:50 – 10:00	Questions and Answers
10:00 – 10:30	Break
10:30 – 11:05	Current State of Oocyte Freezing and Alternative Approaches **Ali Eroglu, D.V.M., Ph.D.**
11:05 – 11:15	Questions and Answers
11:15 – 11:50	Challenges in Ovarian Cryopreservation **Kutluk H. Oktay, M.D.**
11:50 – 12:00	Questions and Answers
12:00 – 13:00	Lunch
13:00 – 13:45	Special Cases of Fertility Preservation: In Estrogen Sensitive Cancer and Children **Kutluk H. Oktay, M.D.**
13:45 – 14:00	Questions and Answers
14:00 – 14:45	Other Options: IVM and Gonadal Suppression? **Lynn M. Westphal, M.D.**

<u>Saturday, October 17, 2009 (continued)</u>

14:45 – 15:00 Questions and Answers

15:00 – 15:30 Break

15:30 – 16:05 Emerging Technologies for the Preservation of Fertility In Female Cancer Patients
Jonathan L. Tilly, Ph.D.

16:05 – 16:15 Questions and Answers

16:15 – 16:50 An Individualized Approach to Fertility Preservation: Marrying Knowledge of Reproductive Biology to the Practice (Case Discussions)
All Faculty

16:50 – 17:00 Questions and Answers

COUNSELING PATIENTS ABOUT REPRODUCTIVE POTENTIAL AFTER CANCER TREATMENT

Lynn M. Westphal, M.D.
Associate Professor
Obstetrics and Gynecology
Stanford University School of Medicine
Stanford, California

LEARNING OBJECTIVES

At the conclusion of this presentation, participants should be able to:

1. Explain the effects of cancer treatment on fertility.
2. Counsel patients about treatment options.
3. Discuss pregnancy after cancer treatment.

Counseling Patients about Reproductive Potential after Cancer Treatment

Lynn M. Westphal, M.D.
Associate Professor
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Disclosure

Lynn M. Westphal

Advisory Board:

- EMD Serono
- Schering Plough
- Ferring

Risk of Cancer

- Estimated that over 692,000 women were diagnosed with invasive cancer in 2008
- More than 10 million cancer survivors alive in the U.S.; >270,00 of those diagnosed under age 21
- Improving cure rates = more survivors able to benefit from fertility preservation

Effect of Cancer Treatment

- Women are born with a finite number of oocytes.
- At puberty,about 300,000 follicles are present.
- Chemotherapy/radiotherapy increase rate of oocyte atresia.
- Premature menopause is common.

Future Fertility: Does It Matter?

"When they told me I had cancer, I didn't cry. When they told me I had a 50% chance of infertility, I cried like a baby."

- Female, 17 years old, diagnosed with Ewing's sarcoma

Parent/Child Survey

Goodwin 2007

- Respondents were classified into risk of infertility based on treatment regimen (classified as low, medium, or high risk).
- Low-risk respondents had undue worry about risk of infertility (41% believed their child was at risk).
- 48% of medium-risk and 90% of high-risk respondents knew of the risk of infertility.
- Less than 50% of the high-risk respondents knew that there was a chance of delayed puberty or early menopause.
- Majority of patients and parents wanted to learn more by talking to a specialist in reproductive medicine/endocrinology (46%), by talking to their oncologist (55%), or by printed materials handed out in the hospital (63%).

"Swimming Upstream"

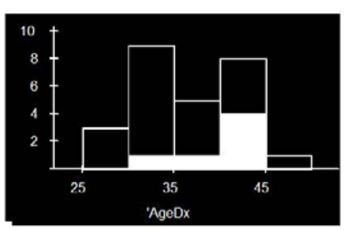
"I brought it (fertility) up to all of them. And they said, "Oh, don't worry about it right now. Right now you need to focus on the tumor and that's it."

-Breast cancer survivor

Information About Risks

...of early menopause / infertility?

- Too little 68% (In black)
- Just right 32% (In white)
- Too much 0%



Would Have Done Chemotherapy?

…If you had been informed well of your risks of menopause and infertility?

- 8/19 Definitely yes
- 6/19 Knew risks and chose chemotherapy
- 4/19 Probably yes
- 1/19 Do not know
- 0/19 Probably not
- 0/19 Definitely not

Concerns About Ovarian Damage

- Young women care deeply about this
  - > 70% response to long mail-in survey!
- Preliminary results
  - Even women over age 40 upset by possible side effects
  - Most report "too little information"
  - Little support for concern about "scaring women away from life-saving chemotherapy"

Risk of Ovarian Failure

- Age
- Chemotherapy regimen
  - Cumulative dose
  - Specific agent

Childhood Cancer Survivor Study

- 2819 survivors of childhood cancer and 1065 sibling controls
- Premature menopause 8% for survivors versus 0.8% for siblings

Effect of Age/Dose

Shalet , 1980

Total average dose of cyclophosphamide resulting in amenorrhea:

- 5.2 g for women age > 40
- 9.3 g for women age 30-39
- 20.4 g for women age 20-29

Alkylating Agents

Highest risk of primordial follicle death

Noncell-cycle specific

Destroy follicles in dose-dependent manner, but can cause damage at low doses

Most commonly used agent is cyclophosphamide

Risk of Cytotoxic Drugs

Adapted from Sonmezer and Oktay , Hum Reprod Update, 10:251,2004

High Risk	Medium Risk	Low Risk
Cyclophosphamide	Cisplatin	Vincristine
Ifosfamide	Adriamycin	Methotrexate
Nitrogen mustard	Carboplatin	Actinomycin D
Busulfan		5-fluorouracil
Melphalan		Bleomycin
Procarbazine		
Chlorambucil		

Probability of Menopause after Chemotherapy

Goodwin 1999

Looked at risk of menopause one year after chemotherapy for breast cancer.
Mean age of women who became menopausal was 3 years greater than those who continued to have menstrual function (45.5 versus 42.3 years).

Ovarian Recovery

Sanders 1988

- BMT before age 25:
  - 10 Gy single TBI — 2/36 recovery
  - 12 Gy fractionated TBI — 7/29 recovery
  - 15.7 fractionated TBI — 0/11 recovery
  - Cylophosphamide 200 mg/kg — 27/27 recovery
- BMT after age 25:
  - All TBI exposures — 0/68 recovery
  - Cyclophosphamide 200 mg/kg — 5/16 recovery

BMT = bone marrow transplant; Gy = gray; TBI = total body irradiation

Radiotherapy and Ovarian Function

- LD_{50} < 2 Gy (Wallace, 2003)
- Ovarian failure in 97% of females following whole abdominal radiation (20-30 Gy) in childhood (Wallace, 1989)
- Ovarian failure in 90% of women after total body irradiation (9.2-15.75 Gy) (Sanders, 1996)

LD_{50} = median lethal dose

Radiotherapy and Ovarian Failure

Wallace 2005

- Ovarian failure varies by age:

20.3 Gy at birth

18.4 Gy at age 10

16.5 Gy at age 20

14.3 Gy at age 30

Radiotherapy and Uterine Function

- Injury to uterine vasculature
- Reduced elasticity and volume of uterus
- Increase in pregnancy complications:
  - Spontaneous abortion
  - Preterm labor
  - Fetal distress
  - Low birth weight

Uterine Restoration?

Letur-Konirsch 2002

- Pelvic radiation can induce uterine dysfunction.
- Evaluated 6 women with childhood cancers and radiation damage to the uterus (20-40 Gy to pelvis)
- Pentoxifylline and vitamin E given for 12 months
- Significant improvement in endometrial thickness, myometrial dimensions, and diastolic uterine artery flow

Methods for Fertility Preservation

- Cryopreservation of oocytes
- Cryopreservation of embryos
- Cryopreservation of ovarian tissue
- Cotreatment with gonadotropin-releasing hormone (GnRH) agonists
- Chemotherapy with less ovarian toxicity
- Conservative surgery
- Oophoropexy

Oophoropexy

- Transpose ovaries out of pelvis (just prior to radiation)
- Success variable
- Failure from scatter radiation, vascular compromise, age of patient, radiation dose, ovarian “migration”
- Spontaneous pregnancy can occur.

Stimulation of Cancer Patients

Quintero 2008

	Controls (50)	Cancer Subjects (50)	P-value
Days of stimulation	9	10.5	<.001
Total amount of gonadotropins	3416 IU	4174 IU	.003
Number of oocytes retrieved	11.5	13	N/S

NS = not statistically significant

Timing of Cryopreservation

Madriano, 2007

- Mean time from definitive surgery to initiation of chemotherapy:
  46.8 days
- Mean time interval from evaluation to retrieval:
  33.3 days (10-65 days)

Gynecologic Cancers

21% occur in reproductive age:

- Cervical
- Ovarian
- Uterine

Ovarian Cancer

May consider conservative management:

- Early invasive epithelial ovarian carcinoma
- Low malignant potential
- Malignant germ cell

Pregnancy after Epithelial Ovarian Carcinoma

	Pregnancy rate
Colombo (1994)	25/25 (100%)
Zanetta (1997)	20/36 (56%)
Duska (1999)	2/6 (33%)
Morice (2001)	4/18 (22%)
Schilder (2002)	17/24 (71%)

Conservative Treatment of Endometrial Carcinoma

	n	Treatment	Duration	Regression	Recurrence	Pregnancy
Kim,1997	7	Megestrol acetate	3 months	4 (57%)	2 (50%)	0
Kim,1997	14	Megestrol acetate or MPA	Up to 1 yr	9 (64%)	1 (11%)	3 (14%)
Randall,1997	12	Megestrol acetate or MPA	3-18 months	9 (75%)	1 (11%)	3 (25%)
Kaku,2001	12	MPA	2-14 months	9 (75%)	2 (22%)	2 (16%)
Wang,2002	9	Megestrol acetate +/-Tam	N/A	8 (89%)	4 (50%)	4 (44%)
Gotleb,2003	13	Megestrol acetate or MPA	3.5 months	13 (100%)	6 (46%)	3 (23%)
Jadoul,2003	5	GnRH agonist	3-6 months	5 (100%)	N/A	4 (80%)

MPA = medroxyprogesterone acetate; Tam = tamoxifen

Outcomes of Trachelectomy

Covens, 2003

Number of patients	81
Median follow-up time	30 months
Recurrences	5
Attempting conception	37
Pregnancies	22
Live births	18
Delivery before 36 weeks	6

Male Fertility Preservation

- First births from cryopreserved semen reported in 1953 (Bunge and Sherman).
- Male gonad is very susceptible to effects of chemotherapy.
- Semen cryopreservation before chemotherapy/radiotherapy is relatively simple.
- Intracytoplasmic sperm injection (ICSI) allows even very poor samples to be used.

TESE/ICSI in Azoospermic Men Postchemotherapy

Chan 2001

- 17 men azoospermic after chemo for range of malignancies
- 20 attempts of testicular sperm extraction (TESE)
- Sperm retrieved in 9 cases
- Biochemical pregnancy in 4 of 9 couples
- Live delivery in 2 of 9 patients

Preimplantation Genetic Diagnosis

- Fear of transmission of disease can affect reproductive decisions.
- Has been performed for common syndromes of predisposition to breast, ovarian cancers (BRCA1, BRCA2)
- Many other inherited cancer predispositions (e.g., Li-Fraumeni, retinoblastoma, neurofibromatosis, multiple endocrine neoplasia [MEN])

Assessing Ovarian Reserve

- Characterizing effects of chemotherapy difficult
- Van Beek (2007) showed that anti-müllerian hormone (AMH) was most sensitive predictor in Hodgkin's disease patients. Most women who reported a pregnancy had normal AMH levels at time of study.
- Bath (2003) found early follicular follicle-stimulating hormone (FSH) higher, AMH lower and ovarian volume smaller in cancer survivors.

Pregnancy Outcomes after Cancer

Green 2002

- 1915 females diagnosed with cancer at age <21 years
- 4029 pregnancies (63% live births, 1% stillbirth, 15% miscarriage, 17% abortion, 3% unknown)
- No significant differences in patients who received chemotherapy and controls
- Pelvic irradiation: lower birth weight

Pregnancy after Radiation/BMT

Wang 1998

- Diagnosed with acute myeloid leukemia (AML) at age 16
- Treated with cyclophosphamide(120mg/kg) and TBI (1575 cGy in 7 fractions)
- Became amenorrheic; 5.5 years after BMT, FSH=116
- Started to have irregular menses; conceived at age 22 and delivered healthy term infant

Breast Cancer

- Most common cancer in reproductive-age women
- About 16,000 of the estimated 182,000 cases/year will occur in women under age 45 years.

Pregnancy after Breast Cancer

Blakely 2004

- 383 women <35 years old when diagnosed with breast cancer
- 47 (13%) had at least one pregnancy after treatment
- Recurrence 23% for those who became pregnant; 54% for those who did not conceive
- Patients with a pregnancy tended to have earlier stage disease (stage I/II: 80% vs 73%)
- Correlates with other studies that have shown no increase in recurrence risk

Safety of Pregnancy after Chemotherapy

- In general, pregnancy outcomes in cancer survivors have shown no increase in birth defects.
- Higher risk of cancer in offspring with inherited cancer gene mutations(Wilm's tumor, retinoblastoma)
- Higher risk of poor pregnancy outcomes after abdominal/total body irradiation

Other Fertility Options

- Ovum donation
- Gestational surrogacy
- Sperm donation

American Society for Clinical Oncology (ASCO) Recommendations

Lee 2006

"Oncologists should address the possibility of infertility with patients treated during their reproductive years and… refer appropriate and interested patients."

- http://www.asco.org/guidelines/fertility

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NOTES

NOTES

PRACTICAL CRYOBIOLOGY

Ali Eroglu, Ph.D.
Associate Professor
Institute of Molecular Medicine and Genetics
Medical College of Georgia
Augusta, Georgia

LEARNING OBJECTIVES

At the conclusion of this presentation, participants should be able to:

1. Discuss the basic principles of cryobiology.
2. List the different steps of cryopreservation techniques.
3. Describe the major modes of cryoinjury.
4. Explain the proper handling of cryopreservation samples.

Practical Cryobiology

Ali Eroglu, Ph.D.
Associate Professor

Institute of Molecular Medicine and Genetics
Medical College of Georgia, Augusta, GA

ASRM Post Graduate Course 2009

Learning Objectives

At the conclusion of this presentation, participants should be able to:

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- ❑ List the different steps of cryopreservation techniques.
- ❑ Describe the major modes of cryoinjury.
- ❑ Explain the proper handling of cryopreservation samples.

Disclosure

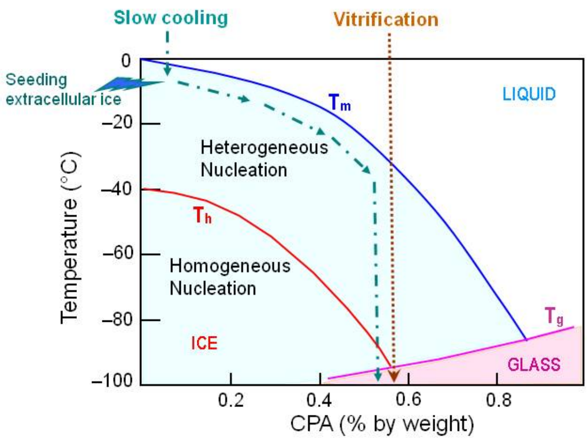
Ali Eroglu, Ph.D.

Stockholder in Gamete Technology, Inc.

Cryopreservation Techniques

- Slow cooling
  - Moderate concentrations (e.g., 1.5 M) of penetrating cryoprotectants (CPAs)
  - Deliberate seeding of extracellular ice
- Vitrification
  - High concentrations (e.g., 6 M) of penetrating cryoprotectants
  - Avoidance of any ice formation

Cryopreservation Techniques



Common Steps of Cryopreservation Techniques

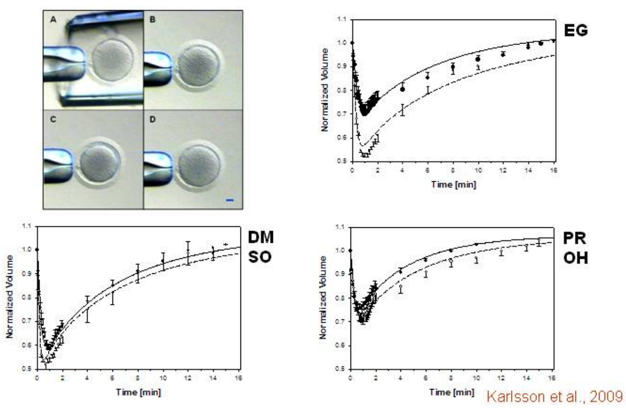
1. Loading/adding of cryoprotectants
2. Cooling to LN2-temperature
3. Warming of the samples
4. Removal/dilution of cryoprotectants

Common Steps of Cryopreservation Techniques

1. Loading/adding of cryoprotectants

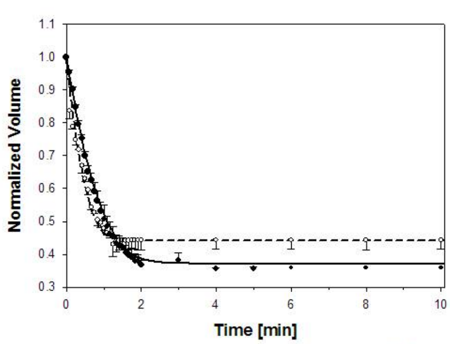
- Possible injuries: Osmotic shock, chemical toxicity, parthenogenetic activation of oocytes, depolymerization of the cytoskeleton, polyploidy, premature exocytosis of cortical granules and zona hardening

Permeability of Rhesus Monkey Oocytes to Common Cryoprotectants



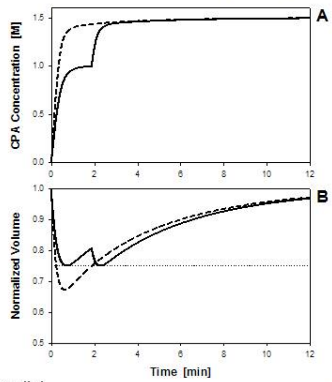
Karlsson et al., 2009

Volumetric Response of Rhesus Monkey Oocytes to Hypertonic PBS



Karlsson et al., 2009

Optimization of Cryoprotectant Loading (PrOH) into Rhesus Monkey Oocytes



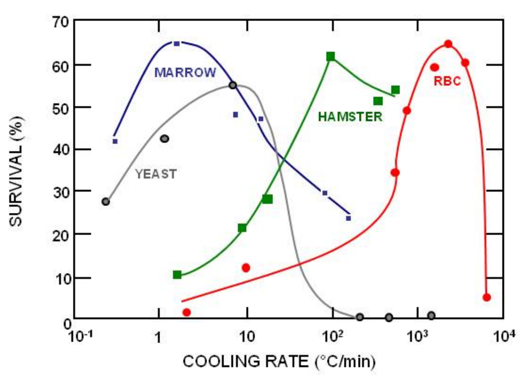
PrOH = 1,2-propanediol

Karlsson et al., 2009

Common Steps of Cryopreservation Techniques

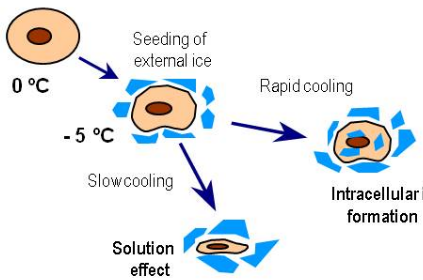
2. Cooling to liquid nitrogen (LN_2) temperature
   - Possible injuries: Chilling injury, intracellular ice formation (IIF), solution effect injury, membrane lipid phase change

Survival Signatures



Redrawn from Mazur et al., 1970

Two-Factor Hypothesis of Freezing Injury

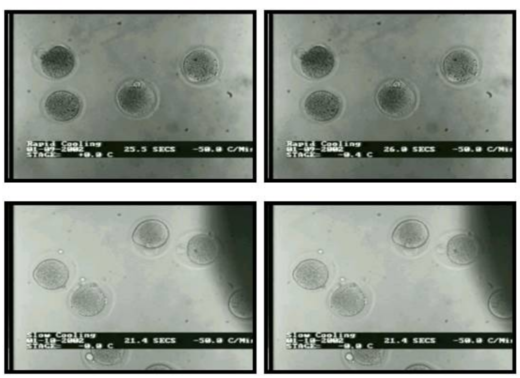


Redrawn from Mazur, 1977

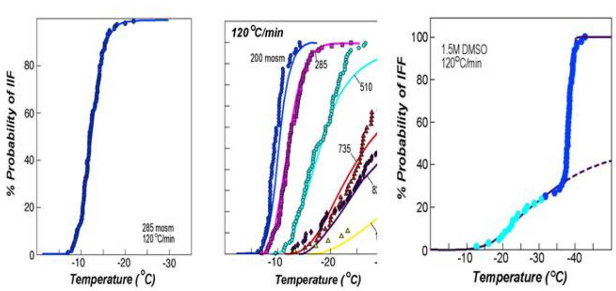
Cryomicroscopy of Mouse Oocytes

Rapid cooling

Slow cooling

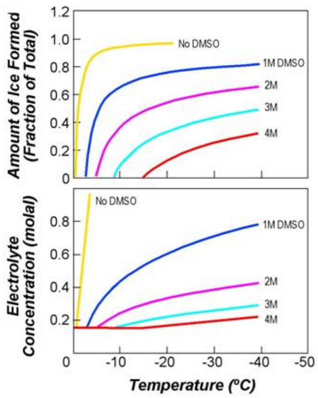


Effect of Dehydration and Cryoprotectant on Intracellular Ice Formation (IIF)



Redrawn from Toner et al., 1991

Amount of Ice and Electrolyte in Frozen DMSO Solution



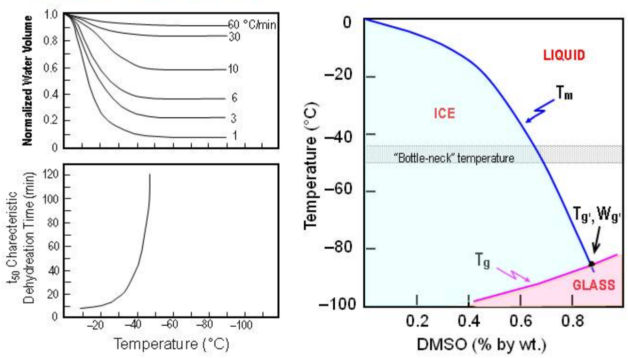
DMSO = dimethylsulfoxide

Redrawn from Pegg et al., 1987

"Bottle-neck" to Cellular Dehydration

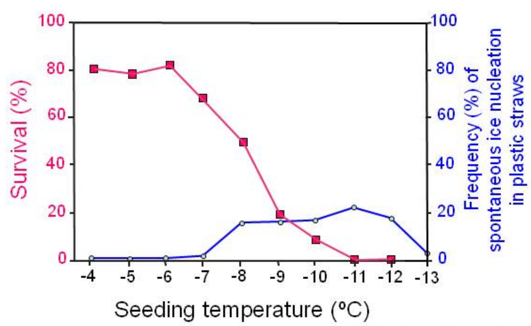
(Redrawn from Karlsson et al, 1994)

Phase Diagram of DMSO



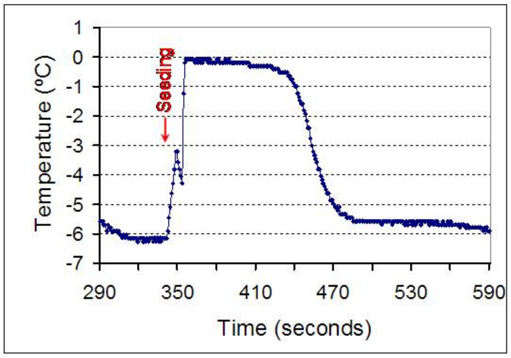
Effect of the Ice Seeding Temperature on the Survival of Mouse Embryos

The embryos were slowly cooled to -80°C before being transferred to LN_2 and subsequently slowly thawed at ~ 6°C/minute.



Redrawn from Whittingham 1977

Seeding of Extracellular Ice



Common Steps of Cryopreservation Techniques

3. Warming of the samples

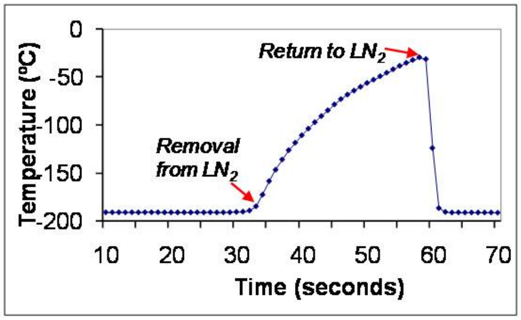
- Possible injuries: Thermal stresses and physical damage (e.g., zona cracking), osmotic shock

Warming Rates of Cryopreserved Samples

- Vitrification
  - As fast as possible to avoid devitrification while attempting to reduce thermal stresses
- Slow cooling
  - Depends on the cooling rate and plunge temperature
    - Standard slow cooling (cooling to -35ºC at 0.3ºC/minute and then plunging into LN_2): Moderately rapid warming (200-350 ºC/minute)
    - Slow cooling below -60ºC and then plunging into LN_2): Slow warming at <25 ºC/minute)

Warming of a ¼cc Plastic Straw in Air for 30 Seconds

- To reduce thermal stresses and thus zona cracking
- To allow evaporation of LN_2 if leaked

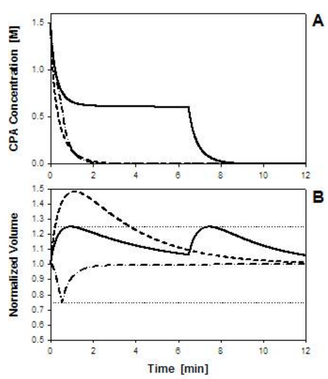


Common Steps of Cryopreservation Techniques

4. Removal/dilution of cryoprotectants

- Possible injuries: Osmotic shock, chemical toxicity, parthenogenetic activation of oocytes, depolymerization of the cytoskeleton, polyploidy, premature exocytosis of cortical granules and zona hardening

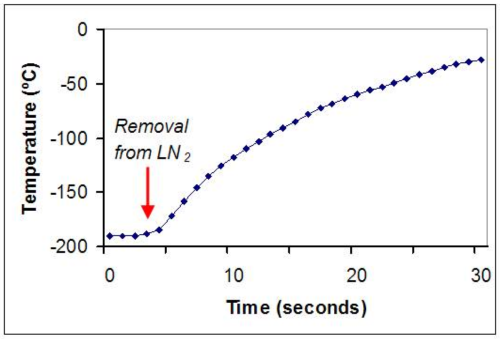
Optimization of Cryoprotectant Removal (PROH) from Rhesus Monkey Oocytes



Karlsson et al., 2009

Handling of Frozen Samples

¼cc plastic straw containing 150 µL fluid



Safety Issues

- ❑ Avoiding contamination of storage tanks and cross-contamination of samples
- ❑ Routine monitoring of LN_2 level, visually and using electronic devices
- ❑ Splitting samples into 2 or more storage tanks
- ❑ Proper labeling and identification of samples
- ❑ And …

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NOTES

CURRENT STATE OF OOCYTE FREEZING AND ALTERNATIVE APPROACHES

Ali Eroglu, Ph.D.
Associate Professor
Institute of Molecular Medicine and Genetics
Medical College of Georgia
Augusta, Georgia

LEARNING OBJECTIVES

At the conclusion of this presentation, participants should be able to:

1. Summarize the basics of cryobiology.
2. Discuss applications of stem cells to oncology.
3. List the major modes of cryoinjuries.
4. Describe the basics of cancer stem cell hypothesis.

Current State of Oocyte Freezing and Alternative Approaches

Ali Eroglu, Ph.D.
Associate Professor

Institute of Molecular Medicine and Genetics
Medical College of Georgia, Augusta, Georgia

Learning Objectives

At the conclusion of this presentation, participants should be able to:

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- ❑ List the major modes of cryoinjuries.
- ❑ Describe the basics of cancer stem cell hypothesis.

Disclosure

Ali Eroglu, Ph.D.

Stockholder in Gamete Technology, Inc.

Slow Cooling

The first successful cryopreservation mouse embryos:
Whittingham, Leibo & Mazur, Science 178: 411, 1972

The first successful cryopreservation human embryos:
Trounson and Mohr, Nature 305:707, 1983

Both studies utilized the same slow cooling protocol.

Vitrification

First studies:

Luyet 1937, Biodynamica 1; 1-7

Successful mouse embryo vitrification

Rall and Fahy, Nature 313; 573-75, 1985

Successful vitrification of drosophila embryos
Steponkus et al., Nature 345; 170-72, 1990
Mazur et al., Science 258; 1932-35, 1992

Minimum volume, faster cooling
Riha et al., Zivoc Viroba 36 113-20, 1994
Martino et al., Biol Reprod 54;1059-69, 1996

Potential Applications of Oocyte Cryopreservation

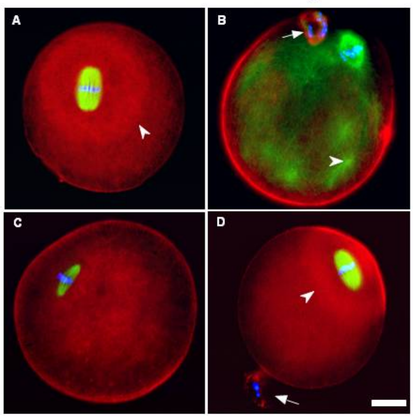
- Preservation of future fertility of women anticipating loss of ovarian function
- Preservation of excess number of oocytes in IVF/ET programs
- Stopping biological clock
- Conservation of genetic material of endangered species and transgenic animals
- Agricultural (livestock breeding) and research applications

IVF/ET = in vitro fertilization/embryo transfer

Cryoinjuries to Oocytes

- Intracellular ice formation
- Solution effect
- Chemical toxicity of common cryoprotectants
- Osmotic shock
- Chilling injury
- Premature exocytosis of cortical granules and zona hardening
- Disruption of the oocyte cytoskeleton and spindle microtubules
- Parthenogenetic activation
- Polyploidy

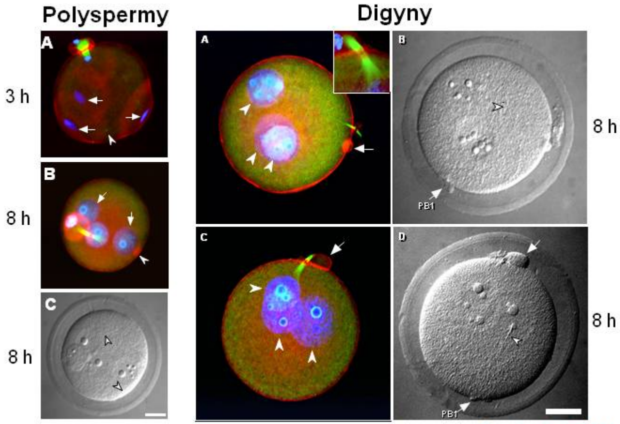
Post-thaw Recovery



Eroglu et al.,1998

Polyploidy After Cryopreservation at the M II Stage

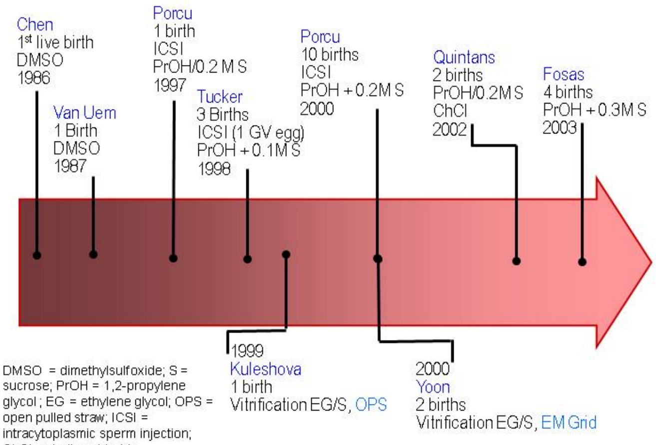
Polyspermy **Digyny**



M II = metaphase II

Eroglu et al.,1998

Human Oocyte Cryopreservation: Some Key Milestones



DMSO = dimethylsulfoxide; S = sucrose; PrOH = 1,2-propylene glycol; EG = ethylene glycol; OPS = open pulled straw; ICSI = intracytoplasmic sperm injection; ChCl = choline chloride

Live Birth Outcomes: Slow Cooling/No Sucrose

- Two early studies:
  Chen (1986) – rapid thaw
  van Uem et al. (1987) – slow thaw
  - Both used infertile cohort and whole cumulus oocyte complex frozen
  - DMSO
  - Conventional insemination procedure

Year	Author	Survival (%)	Fert (%)	Infants	Oocytes/ Infant
1986	Chen	32/40(80)	25/30(83)	2	20
1987	Van Uem	7/28(25)	2/4(50)	1	28
				3	23

Cumulative Success Rates Using Slow Cooling + Sucrose

Variable	1.5 M PrOH + 0.1 M sucrose	1.5 M PrOH + 0.2 M sucrose	1.5 M PrOH + 0.3 M sucrose
Survival, % (no. of thawed oocytes)	50 (3537)	72 (926)	74 (4902)
Fertilization (ICSI), %	54	80	73
Cleavage, %	85	93	90
Embryos per 100 thawed oocytes	23	53	49
Implantation rate, %	10	17	5
Implantations per 100 thawed oocytes	2.3	9.1	2.4

Adapted from Gook & Edgar 2007

Success Rates Using Donor Oocytes and Slow Cooling + 0.3 M Sucrose

Mean age of donors	28.3
Total number of cryopreserved oocytes	79.0
Total number of thawed oocytes	79.0
Mean survival rate	86.1 %
Mean fertilization rate	89.7 %
Mean cleavage rate	91.8 %
Pregnancy rate per transfer	75.0 %
Implantation rate	26.1 %

Barrit et al.,2007

Slow Cooling Using Sodium-Depleted, Choline-Supplemented Media

Replacement of sodium chloride (NaCl) with choline chloride improves the outcome of mouse oocyte cryopreservation (Stachecki et al., 1998).

Author	Survival (%)	Fertilization (%)	Cleavage (%)	Pregnancy (%)	Births
Quintans 2002	58/109 (53)	33/58 (57)	33/33 (100)	5/12 (42)	2
Boldt 2003	67/90 (74)	39/66 (59)	33/39 (85)	4/11 (36%)	5
Petracco 2006	99/158 (63)	61/99 (62)	56/61 (92)	4/16 (25)	5
Boldt 2006	218/361 (60)	134/216 (62)	110/134 (82)	14/43 (33)	9

Cumulative Success Rates of Slow Cooling Between 1996 and 2005

Variable	Outcome (all cycles reported as of March 2006)
Age, mean ± SE	33.7
Fertilization rate	64.9 (2,478/3,818)
Clinical pregnancies per transfer	20.6 (153/742)
Implantation rate	10.1 (185/1,828)

Adapted from Oktay et al., 2006

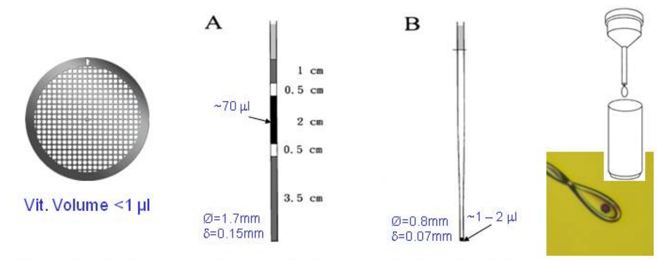
Cumulative Success Rates of Slow Cooling Between 2006 and 2008

Total number of cryopreserved oocytes	7439
Total number of thawed oocytes	3991
Mean survival rate	75.7%
Mean fertilization rate	77.6%
Mean cleavage rate	89.2%
Pregnancy rate per transfer	22.3%
Implantation rate	10.5%
Abortion rate	17.3%

Borini et al., 2006; Levi Setti et al., 2006; Chamayou et al., 2006; De Santis et al., 2007; Bianchi et al., 2007, Barrit et al., 2007; Parmegiani et al., 2008

Vitrification: Minimizing Volume, Increasing Cooling Rate

EM GRID	STRAW	OPEN PULLED STRAW	CRYO-LOOP



EM GRID	STRAW	OPEN PULLED STRAW	CRYO-LOOP
~9,000 to 30,000°C/min Yoon (2000)	~ 2,000 to 4,000°C/min Kuleshova (1999)	~5,300 to 10,300°C/min Vajta (1997)	-- Lane (1999)

Fertilization and Pregnancy Results After Vitrification

Variable	Reports before June 2005	Reports after June 2005
Age, mean ± SE	32.3 ± 0.85	32.3
Fertilization rate	70.6% (156/221)	75.4% (481/638)
Clinical pregnancies per thawed oocytes	2% (10/503)	6% (51/851)
Live births per thawed oocytes	2% (10/503)	4.6% (39 [7]/851)
Clinical pregnancies per transfer	29.4% (10/34)	51% (51/100)
Live births per transfer	29.4% (10/34)	39% (39 [7]/100)
Implantation rate	8.8% (12/137)	20.5% (69/336)

Data in square brackets are number of ongoing pregnancies.

Adapted from Oktay et al., 2006

Cumulative Success Rates of Vitrification Between 2006 and 2008

Total number of vitrified oocytes	3164
Total number of warmed oocytes	1709
Mean survival rate	88.0%
Mean fertilization rate	82.7%
Mean cleavage rate	75.6%
Pregnancy rate per transfer	51.3%
Implantation rate	26.2%
Abortion rate	15.2%

Selman et al., 2006; Antinori et al., 2007; Yoon et al., 2007; Chian et al., 2008; Keskintepe et al., 2008; Sher et al., 2008; Cobo et al., 2008

Comparison of Success Rates of Slow Cooling and Vitrification

	Slow cooling	Vitrification
Total number of vitrified oocytes	7439.0	3164
Total number of warmed oocytes	3991.0	1709
Mean survival rate	75.7%	88.0%
Mean fertilization rate	77.6%	82.7%
Mean cleavage rate	89.2%	75.6%
Pregnancy rate per transfer	22.3%	51.3%
Implantation rate	10.5%	26.2%
Abortion rate	17.3%	15.2%

Data obtained from papers published between 2006 and 2008

Problems with Reporting Results and Comparison

- ❑ Age of patients
- ❑ Routine, large volumes vs. carefully controlled donor studies
- ❑ Selection of oocytes and resulting embryos vs. non-selection
- ❑ Missing data points
- ❑ Lack of appropriate controls
- ❑ Comparison studies: generalization issue and expertise in tested techniques

Advantages and Disadvantages

Slow cooling

PROS
- Closed system prevents contamination (better biosafety)
- Lower cryoprotective agent (CPA) concentrations, less CPA toxicity
- Better safety margin
- Volume

CONS
- Slow (~2 to 3 hours)
- Controlled-rate freezer needed
- Prone to chilling injury

Vitrification

PROS
- Fast (~15 to 30 minutes); however, small number of oocytes can be frozen at a time
- No expensive equipment needed
- Minimizing chilling injury

CONS
- High CPA concentrations and related increased toxicity and osmotic stress
- Volume
- Training
- Safety margin
- Biosafety
- Prone to devitrification

Biosafety Risk for Open Vitrification Systems

- ❑ Documented cases of LN_2-mediated disease transmission
  - Tedder et al., 1995
  - Fountain et al., 1997
  - Berry et al., 1998
- ❑ Experimental demonstration by Bielanski et al. (2000) using OPS

Conclusions

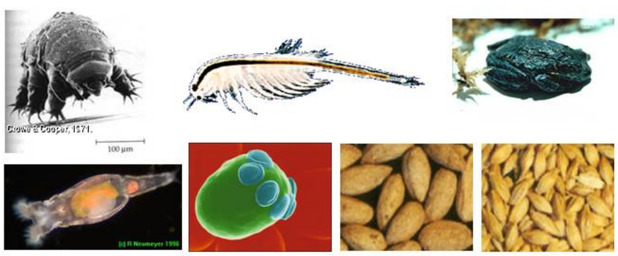
- ❑ The overall success rate of human oocyte cryopreservation has been significantly improved in recent years. Several factors seem to contribute to this:
  - Better understanding of fundamental cryobiology of human oocytes
  - Better quality of oocytes
  - Increased public and patient awareness
  - More clinical experience with oocyte cryopreservation and thus more experienced practitioners

Conclusions (Continued)

- Both approaches have advantages and disadvantages.
- While both slow-cooling and vitrification approaches have been improved over the years, some concerns remain to be addressed.
- Further research is needed to develop more reliable and safe cryopreservation techniques,

Alternative Approaches

In nature, sugars play a key role in survival of extreme conditions (e.g., drought, freezing).

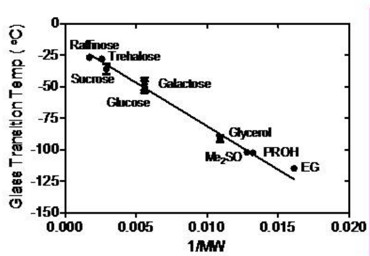


Hypothesis:

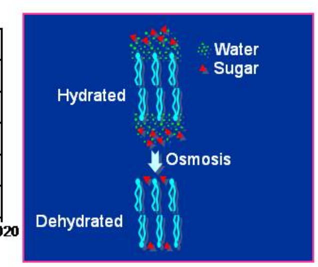
When present both intra- and extracellularly, sugars may protect mammalian oocytes against freezing-associated stresses.

Mechanisms of Protection Afforded by Trehalose

Vitrification hypothesis

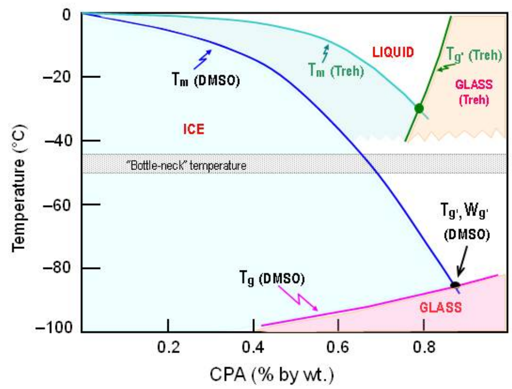


"Water replacement" hypothesis

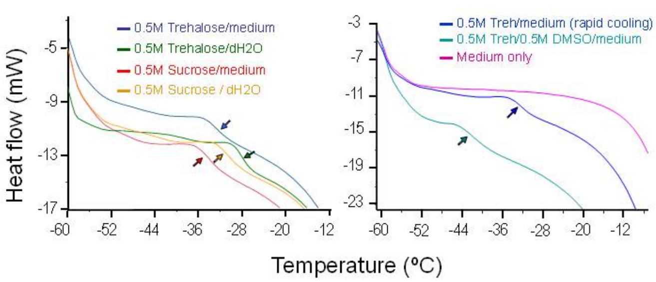


Adapted from Eroglu 2009

Comparison of Phase Diagrams: DMSO vs. Trehalose



Glass Transition Temperature of Freeze-Concentrated Sugars



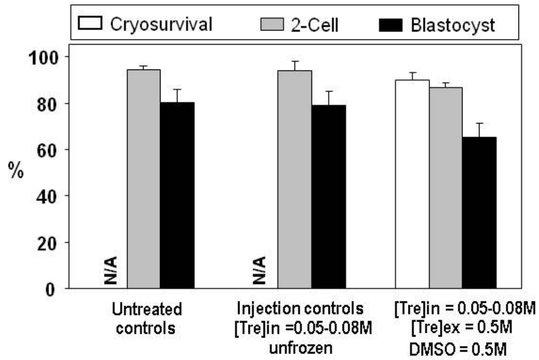
Adapted from Eroglu et al.,2009

Trehalose as a Multifunctional Molecule

- ❑ Antioxidant
- ❑ Chemical chaperone
- ❑ Osmolyte
- ❑ Cryoprotectant

- Disaccharide trehalose inhibits bone resorption in overiectomized mice. *Nishizaki et al., Nutr. Res. 2000*
- Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Tanaka et al., Nature Medicine, 2004.*
- Trehalose, a Novel mTOR-independent Autophagy Enhancer, Accelerates the Clearance of Mutant Huntingtin and -Synuclein. *Sarkar et al., J. Biol. Chem. 2007.*
- Trehalose reduces aggregate formation and delays pathology in a transgenic mouse model of oculopharyngeal muscular dystrophy. *Davies et al., Human Molecular Genetics 2006.*

Survival, Fertilization, and Embryonic Development of Mouse Oocytes After Cooling to -196 °C



Adapted from Eroglu et al.,2009

Offspring Derived From Trehalose-Injected and Frozen-Thawed Oocytes

First generation

Second generation

	Delivery rate
Control	23%
Cryopreservation	19%

Adapted from Eroglu et al.,2009

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NOTES

NOTES

CHALLENGES IN OVARIAN CRYOPRESERVATION AND TRANSPLANTATION

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Consultant Physician
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LEARNING OBJECTIVES

At the conclusion of this presentation, participants should be able to:

1. Outline the current state of fertility preservation by ovarian cryopreservation and transplantation.
2. Highlight the current limitations of ovarian tissue freezing and transplantation.
3. Apply enhanced ethical considerations when offering ovarian tissue freezing.

Challenges in Ovarian Cryopreservation and Transplantation

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Disclosure

Nothing to disclose

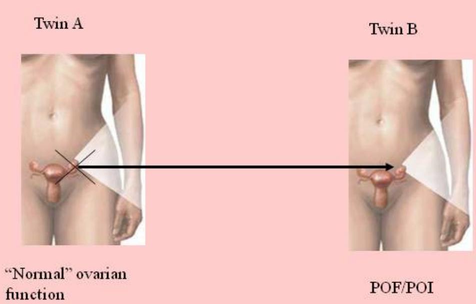
Challenges

- Indications (ethical)-social-twins
- Freezing technique: slow freezing (SF) vs. vitrification (VF); strips vs. whole ovary
- Efficiency: follicle loss after transplant
- Safety (re-seeding cancer/medical risks)
- Transplant techniques: orthotopic vs. heterotopic
- Long-term follow-up/accumulating sufficient numbers

For Social/Elective Indications?

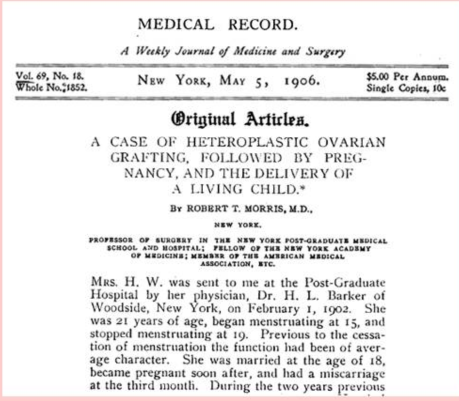
- ≤10 live births from frozen banked tissue
- Mostly women in their late 20s, early 30s
- Even elective oocyte freezing is still controversial
- Too little experience to recommend for social indications
- Restoration of hormonal function an advantage?

Is There a Rationale for Heterologous Ovarian Transplantation?



POF = premature ovarian failure
POI = premature ovarian insufficiency

History of Fresh Heterologous Ovarian Transplant in Twins


MEDICAL RECORD.

A Weekly Journal of Medicine and Surgery

Vol. 69, No. 18. Whole No. 1852. | NEW YORK, MAY 5, 1906. | $5.00 Per Annum. Single Copies, 10c

Original Articles.

A CASE OF HETEROPLASTIC OVARIAN GRAFTING, FOLLOWED BY PREGNANCY, AND THE DELIVERY OF A LIVING CHILD.*

BY ROBERT T. MORRIS, M.D.,

NEW YORK.

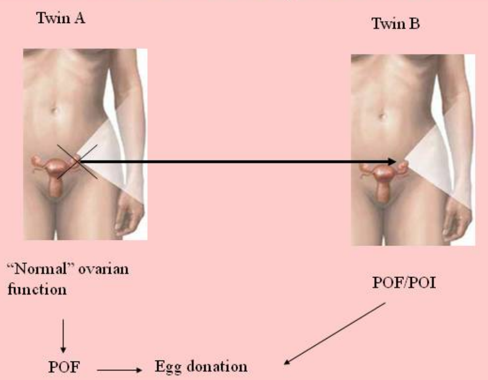
PROFESSOR OF SURGERY IN THE NEW YORK POST-GRADUATE MEDICAL SCHOOL AND HOSPITAL; FELLOW OF THE NEW YORK ACADEMY OF MEDICINE; MEMBER OF THE AMERICAN MEDICAL ASSOCIATION, ETC.

MRS. H. W. was sent to me at the Post-Graduate Hospital by her physician, Dr. H. L. Barker of Woodside, New York, on February 1, 1902. She was 21 years of age, began menstruating at 15, and stopped menstruating at 19. Previous to the cessation of menstruation the function had been of average character. She was married at the age of 18, became pregnant soon after, and had a miscarriage at the third month. During the two years previous

High Concordance for POF and Menopause Between Twins

- **Both monozygotic and dizygotic twins have 3-5 times higher likelihood of POI:**
  - Gosden R et al. Hum Reprod 2007;22:610-615
  - deBruin JP et al. Hum Reprod 16: 2014-2018
  - Snieder H et al. Hum Reprod 1998;83:1875-1880
  - van Asselt KM et al. Fertil Steril 2004;82:1348-1351
  - Do KA et al. Stat Med 2000;19:1217-1235.

Is There a Rationale for Heterologous Ovarian Transplantation?



Twin-Twin Transplantation

- Not feasible if not monozygotic
- Cost/risks of two surgeries
- Spontaneous fertility can be restored
- May have limited use in countries where donor eggs (DE) illegal/not accepted?

Risk of Ovarian Involvement in Cryopreservation Candidates

Low Risk	Moderate. Risk	High Risk
Wilm's tumor	Stage IV breast cancer	Leukemia
Lymphomas	Stage I-III lobular breast cancer	Neuroblastoma
Stage I-III breast cancer (infiltrating ductal)	Adenocarcinoma of cervix	Stage IV lobular breast cancer
Nongenital rhabdomyosarcoma	Colorectal cancer	
Osteogenic sarcoma		
Squamous cell carcinoma of cervix		
Ewing sarcoma		

Sonmezer & Oktay. *Hum Reprod Update*. 2004

Assessing Residual Disease in Ovarian Tissue

- Patients with Hodgkin disease, non-Hodgkin lymphoma (NHL), chronic myeloid leukemia (CML)
- In 2/58, pre-operative imaging showed ovarian involvement
- 0/56 by histology
- 1/56 by molecular markers (CML)
- Risk is low, disease-specific, can be picked up by imaging

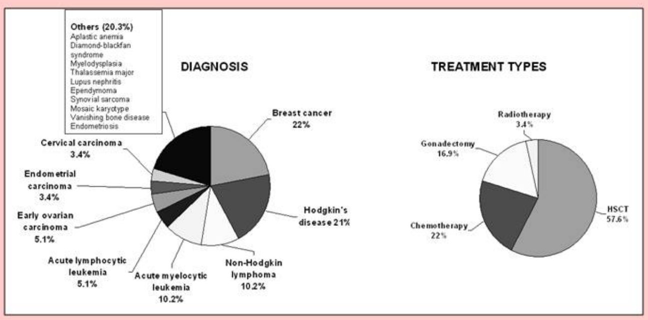
Meirow D 2008 May;23(5):1007-13. Epub 2008 Mar 15.

Safety of Ovarian Cryopreservation in Medically Challenged Females

- 69 cases between May 1997 and March 2009
- Slow freezing with dimethyl sulfoxide (DMSO)
- Age 4 - 44 years (mean 26.7)
- 60 by laparoscopy, 8 during cancer surgery, 1 cesarean section
- No complications (platelet counts as low as 38K)
- No ovarian involvement by histology

Updated from Oktay & Oktem, Fertil Steril, 2008

Cancer and Treatment Type of Patients Undergoing Ovarian Freezing



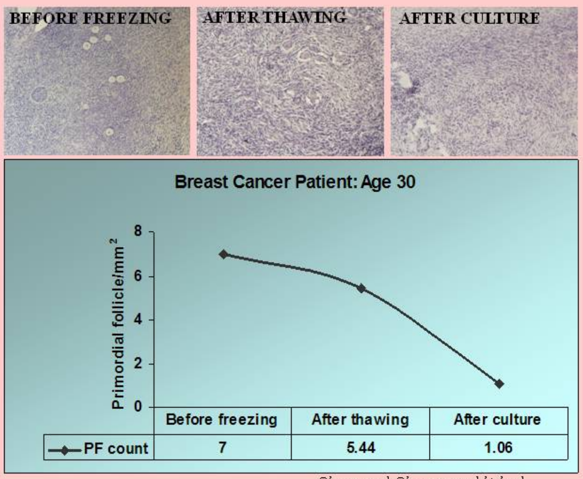
Ovarian Tissue Revascularizes within Days of Grafting

Courtesy of Dr. Roger Gosden

High Rate of Follicle Loss After Transplantation

- Revascularization within 48 hours in rodents
- Vessel maturation lasts for up to 6 days.
  Dissen GA et al. Endocrinology 1994;134:1146-1154
- 5% of primordial follicles lost during freezing and thawing.
- 65% of primordial follicles lost during revascularization (xenografting).
  Baird D et al. Endocrinol 1999;140;462-71.

Baird D et al. Endocrinol 1999;140;462-71.



Oktem and Oktay, unpublished

Does VEGF Improve Ovarian Tissue Survival?

Menstrual cycle characteristics after ovarian transplantation

	No. of monkeys with functioning ovarian transplants	No. of monkeys with one menstrual cycle	No. of monkeys with two consecutive menstrual cycle	Average length of flow (days)	Average cycle length (days)
Sham transplant	0(0)	0(0)	-	N/A	N/A
Transplant with VEGF	5(83)	5(83)	4(67)	3.5	23
Transplant without VEGF	2(40)	4(80)	2(40)	2.7	34
Transplant of cryopreserved tissue	2(50)	2(50)	2(50)	3.2	27

N/A: not applicable
VEGF = vascular endothelial growth factor

Schnorr et al. Hum Reprod 2002;17:612-9.

Whole Ovary Cryopreservation as an Attempt to Improve Follicle Survival

- Successful in mice
- Partial success in sheep:
  - 3/11 patent after 8-10 days

Bedaiwy et al. Fertil Steril 2003;79:594-602.

- Long-term function with new freezing technology:
  - Directional freezing: multithermal gradient (MTG)

Arav A et al. Hum Reprod 2005;20:3554-9.

Whole Ovary Freezing by Directional Freezing in Sheep

- 8 sheep ovaries frozen with MTG technology
- 5/8 grafts survived, but only 2/8 had long-term cyclical function (24-36 months)
- Oocyte retrieval in 2
- Parthenogenic activation

Arav A et al. Hum Reprod 2005;20:3554-9.

Whole Ovary Transplant by MTG?

- 5/8 grafts survived
- In 4, follicles aspirated; in 2, 2oocytes recovered
- In 1, repeat aspiration yielded 4 oocytes
- All activated parthenogenically
- Only 1 remained cyclic by P_4, 36 months later; another had persistent CL

Arav A et al. Hum Reprod 2005;20:3554-9.

P_4 = progesterone
CL = corpus luteum

Whole Ovary Freezing Is Challenging in Human

- Human ovary is larger:
  - 4 x 2 x 0.8 cm (20-35 gm) vs. 2.5 x 1.5 x 0.5 (3-8 gm) in sheep.
- Need to optimize the protocol for germ cells, somatic cells and vascular pedicle.
- No pregnancies reported from whole-ovary freezing yet.

Challenges in Comparing SF vs. VF

- SF is still the "standard" method.
- All pregnancies with SF ovarian tissue
- Literature of VF is hard to evaluate, as most rely on morphology, which does not guarantee function.
- Some used in vitro assessment.

SF vs. VF

- Lower follicle survival and in vitro E_2, P_4 production with 2 different VF methods.

Isachenko V et al. Cryobiology 55 (2007) 261-268

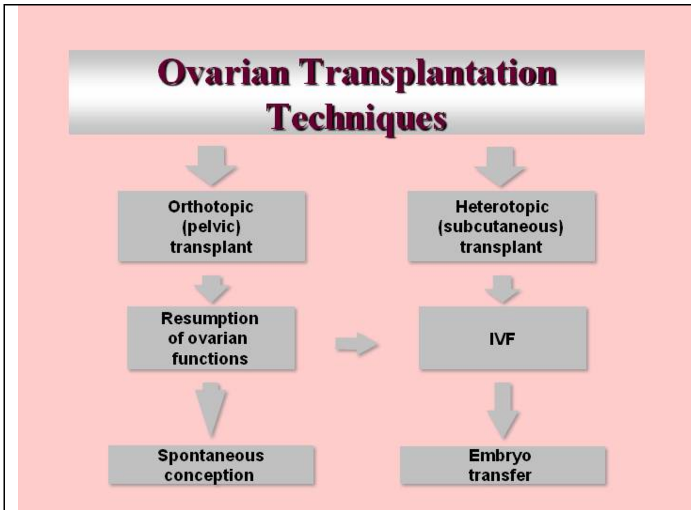
- Similar follicle survival and in vitro E_2, P_4 production?

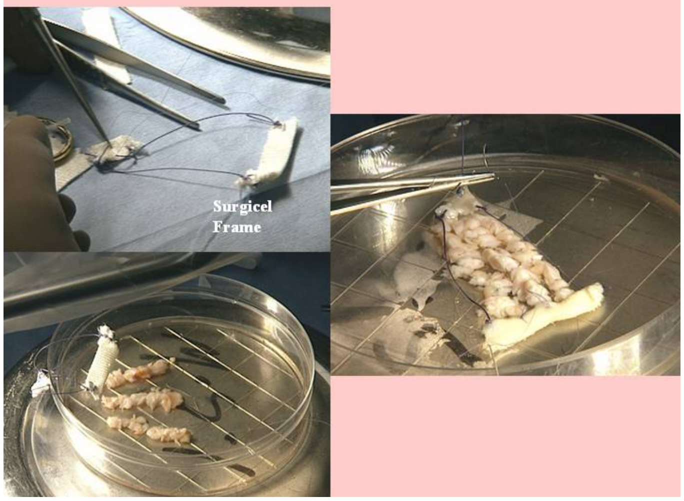
Li YB et al. Chin Med J 120 (2007) 110-114

- Poor survival with VF compared to SF

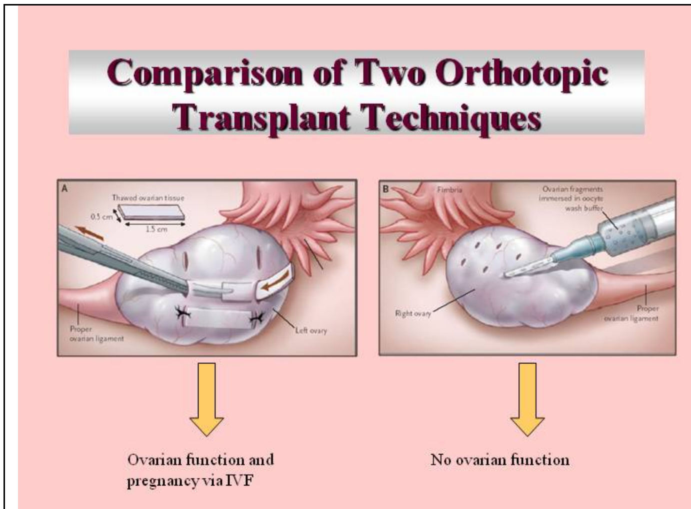
Gandolfi F et al. Fertil Steril 85 (suppl) (2006):1150-1156

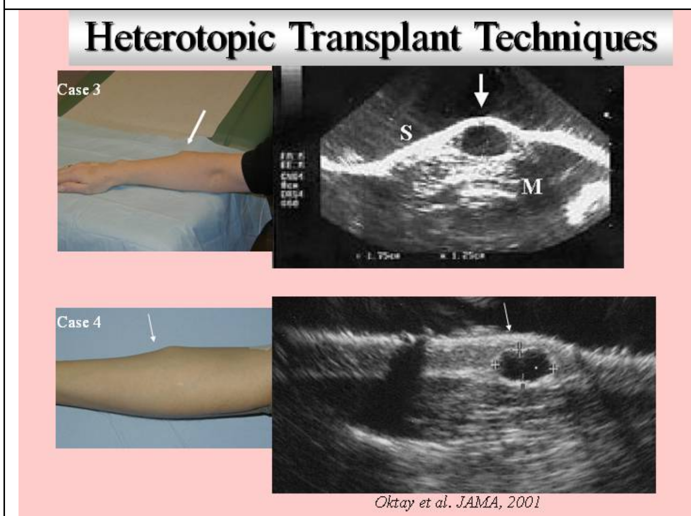
E_2 = estradiol

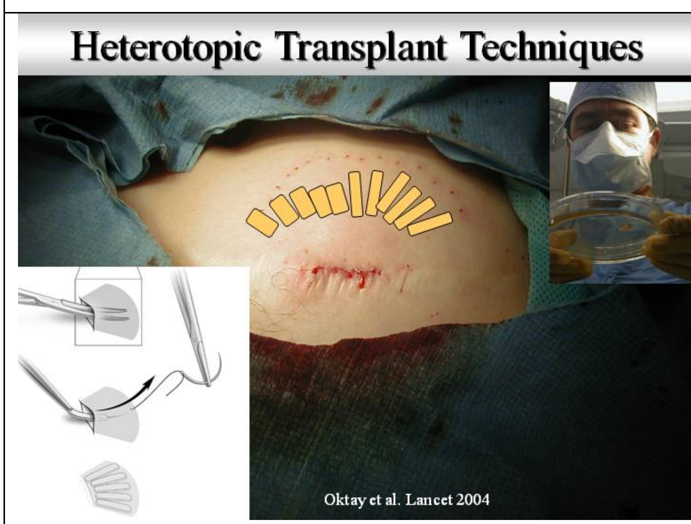

Ovarian Transplantation Techniques
Orthotopic (pelvic) transplant
Heterotopic (subcutaneous) transplant
Resumption of ovarian functions
IVF
Spontaneous conception
Embryo transfer


Surgicel Frame


Reported in Oktay &Karlikaya, NEJM 2000


Comparison of Two Orthotopic Transplant Techniques
A
Thawed ovarian tissue
0.5 cm
1.5 cm
Proper ovarian ligament
Left ovary
B
Fimbria
Ovarian fragments immersed in oocyte wash buffer
Right ovary
Proper ovarian ligament
Ovarian function and pregnancy via IVF
No ovarian function


Heterotopic Transplant Techniques
Case 3
S
M
Case 4
Oktay et al. JAMA, 2001


Heterotopic Transplant Techniques
Oktay et al. Lancet 2004

Heterotopic Transplant

- Non-invasive/repeated procedures feasible
- Can inject agents directly in the graft
- Easy monitoring/removal (risk of recurrence)
- Suitable after pelvic radiation
- Source of pregnancy clear
- Is environment optimal for oogenesis?

Choice of Transplant Technique

	Orthotopic	Heterotopic
Invasiveness	More	Less
Cost	More	Less
Ease of monitoring	Less	More
Animal studies	Delivery in sheep	Blastocyst in sheep Live birth in monkey
Human conception	Live births	4-cell embryo Spontaneous conceptions!
Spontaneous fertility	Yes	No (?)

Difficulties in Assessing Pregnancy Rates After Ovarian Transplantation

- Toxicity of chemotherapy varies
- Is the intact ovary functioning?
- How do we know where the oocyte came from?
- Too few cases to perform controlled studies

Current State of Ovarian Transplant

- Recent meta-analysis (as of June 2008)
- 25 women sought pregnancy after ovarian transplant
- 37% (CI 19, 60) conceived
- Not all were with frozen tissue

Bedaiwy et al. Hum Reprod 2008;23:2709-17

CI = confidence interval

Summary

- There are many ethical and technical challenges remaining before determining the true efficiency of ovarian tissue cryopreservation and transplantation.
- Intense research and discussion is needed.

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endometrial development, ovulation, menstrual patterns and gamete morphology. *Hum Reprod* 2002;17:612-9.

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NOTES

SPECIAL CASES OF FERTILITY PRESERVATION: IN ESTROGEN-SENSITIVE CANCER AND CHILDREN

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LEARNING OBJECTIVES
At the conclusion of this presentation, participants should be able to:

1. Discuss the latest ovarian stimulation techniques in estrogen-sensitive cancer.
2. Describe approaches to fertility preservation in children.
3. Counsel patients and parents regarding fertility preservation options prior to cancer treatments.

Special Cases of Fertility Preservation: In Estrogen-Sensitive Cancer and Children

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Disclosure

- Nothing to disclose

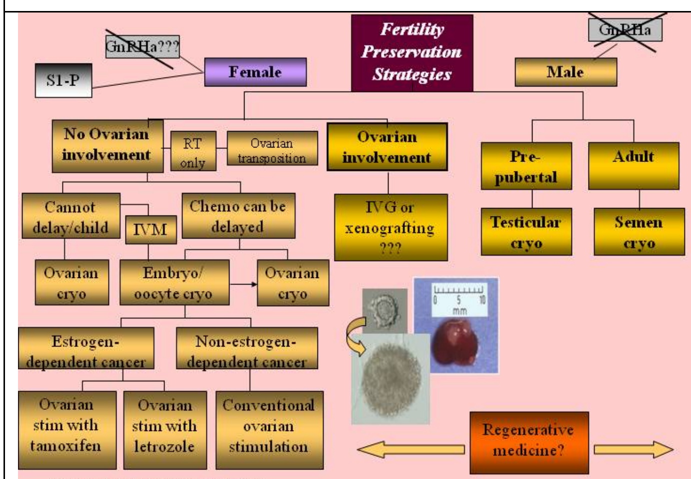
Chemotherapy: The Most Common Cause of Gonadal Failure/Low Reserve?

- Probability of cancer in females :
  - Age 1-39: 1 in 49 (2.03%)
    - Gonadotoxic treatment: 1.1%
  - Age 40-50: 1 in 11 (9.09%)
- Probability of cancer in males:
  - Age 1-39: 1 in 70 (1.42%)
  - Age 40-50: 1 in 12 (8.69%)

Jemal A et al, CA Cancer J Clin 2007

Conditions Commonly Requiring Fertility Preservation

- Breast cancer
- Hematologic malignancies
- Solid tumors: gynecological cancer, gastrointestinal cancer, osteosarcoma, etc.
- Childhood cancers
- Recurrence, relapses, etc.
- Cancer prophylaxis (e.g., BRCA)
- Non-cancer: lupus, hematopoietic stem cell transplant (HSCT), Turner syndrome, benign ovarian tumors, etc.



RT = radiation therapy; IVM = in vitro maturation; IVG = in vitro growth

Oktay & Sonmezer, Human Reproduction, March 2004, Modified

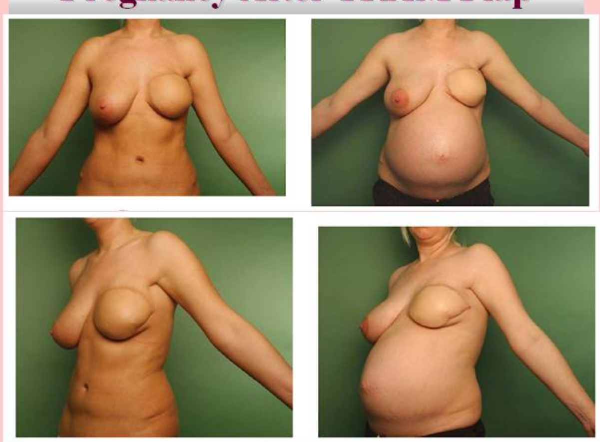
Issues to Consider in Stimulating Cancer Patients

- Timing issues
- Medical issues
  - White blood cell and platelet counts
  - Medications:
    - Anticoagulants
    - Steroids

Points to Consider when Stimulating Cancer Patients

- Most aggressive not necessarily the best
- Cannot take a chance with cancellation of cycle or delay of chemothereapy due to ovarian hyperstimulation syndrome (OHSS)
- Tendency toward infection, blood clots, etc., should be considered
- High risk pregnancy due to past chemotherapy side effects (e.g., cardiomyopathy, pulmonary fibrosis, breast reconstruction)

Pregnancy After TRAM Flap



TRAM = transverse rectus abdominus myocutaneous

Breast Cancer Is the Most Common Indication for Fertility Preservation in the U.S.

- Breast cancer: 35,000
- Hematologic malignancies: 35,000
- Solid tumors: > 10,000
  - Cervical cancer, endometrial cancer, osteosarcoma, etc.
- Childhood cancers: 7,000
- Recurrence, relapses, etc.
- Non-cancer

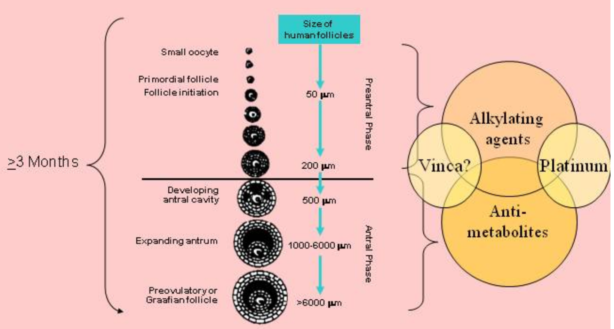
Breast Cancer Is the Most Common Cancer in Women of Reproductive Age

- Affects over 187,000 women/year in the United States[1]
- 25% of cases occur premenopausally[1]
- Adjuvant chemotherapy: ovarian failure
  - CMF: 20-100%; average 68%

CMF = cyclophosphamide, methotrexate, and 5-fluorouracil

1. Facts about breast cancer in the United States. National Alliance of Breast Cancer Organizations Web site.

Different Classes of Agents Affect Different Stages of Follicular Growth



How Reliable Is Menstrual Status to Assess Fertility After Breast Cancer Chemotherapy?

	ACT (n=14)	AC (n=11)	P Value
Age (years)	38.4 ± 5.6	41.4 ± 3.3	NS
Mean time from chemotherapy (months)	38.3 ± 24	37.2 ± 10	NS
Amenorrhea	35.7% (5/14)	9.1% (1/11)	NS
Oligomenorrhea	0% (0/14)	27.3%) (4/11)	0.03
Regular menses + menopausal symptoms	21.4% (3/14)	9.1% (1/11)	NS
All menstrual dysfunction	57.1% (8/14)	54.5% (6/11)	NS
Menstrual dysfunction plus abnormal reserve	77.2%	78.6%	NS

AC = doxorubicin and cyclophosphamide
ACT = AC + taxol
NS = not statistically significant

Reh et al. Fertil Steril 2007

Menstruation Does Not Guarantee Fertility After Breast Cancer Chemotherapy

- 8/11 women with regular menses had abnormal reserve as determined by baseline FSH and E_2

Menstruation is the last event to occur: only the tip of the iceberg!

FSH = follicle-stimulating hormone
E_2 = estradiol

Reh et al. Fertil Steril 2007

"Occult" Impact of Chemotherapy on Fertility in Women with "Normal" Reserve

	Post-chemo (n=22)	Pre-chemo or radiation (n=43)
Mean age	36.5± 0.8	35.7 ± 0.9
Total cycles	38	76
Cycles with retrieval (%)	28 (73.7)	68 (89.4%)
Oocytes retrieved*	4.6 ± 0.9	12.4 ± 7.0
Oocytes fertilized*	2.9 ± 0.7	6.6 ± 4
Live birth /cycle (%) **	7.9	44**
Day 2 AMH (ng/mL)*†	0.27 ± 0.08	0.84 ± 0.3

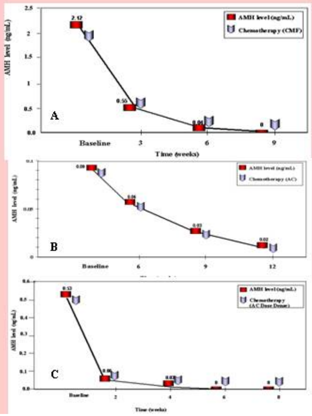
AMH = anti-müllerian hormone

* $p<0.05$

**self for gestational carrier, 25 embryo transfers (ETs)

Compiled from Oktay et al, JCEM 2006 and Azim & Oktay, ASRM abstract, 2006

AMH as a Marker of Chemotherapy-Induced Ovarian Damage



Oktay et al.
J Clin Oncol 2006

JOURNAL OF CLINICAL ONCOLOGY | ASCO SPECIAL ARTICLE

American Society of Clinical Oncology Recommendations on Fertility Preservation in Cancer Patients

Stephanie J. Lee, Leslie R. Schover, Ann H. Partridge, Pasquale Patrizio, W. Hamish Wallace, Karen Hagerty, Lindsay N. Beck, Lawrence V. Brennan, and Kutluk Oktay

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/06/2418-2917/$20.00

A B S T R A C T

Purpose
To develop guidance to practicing oncologists about available fertility preservation methods and related issues in people treated for cancer.

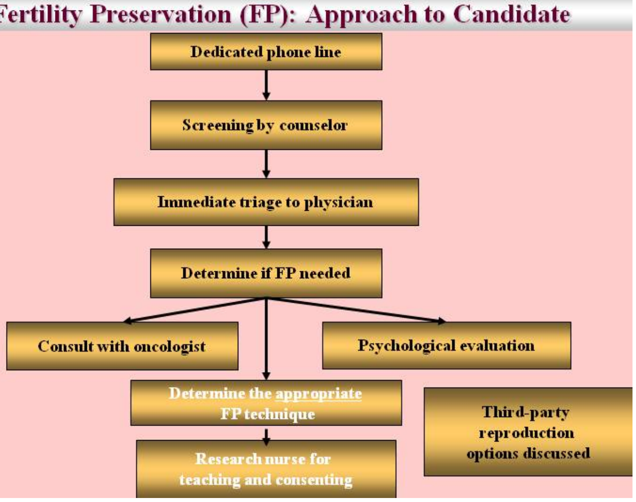
Methods
An expert panel and a writing committee were formed. The questions to be addressed by the guideline were determined, and a systematic review of the literature from 1987 to 2005 was performed, and included a search of online databases and consultation with content experts.

Results
The literature review found many cohort studies, case series, and case reports, but relatively few randomized or definitive trials examining the success and impact of fertility preservation methods in people with cancer. Fertility preservation methods are used infrequently in people with cancer.

Recommendations
As part of education and informed consent before cancer therapy, oncologists should address the possibility of infertility with patients treated during their reproductive years and be prepared to discuss possible fertility preservation options or refer appropriate and interested patients to reproductive specialists. Clinician judgment should be employed in the timing of raising this issue, but discussion at the earliest possible opportunity is encouraged. Sperm and embryo cryopreservation are considered standard practice and are widely available; other available fertility preservation methods should be considered investigational and be performed in centers with the necessary expertise.

Conclusion
Fertility preservation is often possible in people undergoing treatment for cancer. To preserve the full range of options, fertility preservation approaches should be considered as early as possible during treatment planning.

Fertility Preservation (FP): Approach to Candidate



Reasoning for Fertility Preservation in Very Young Breast Cancer Patients

30-year-old
↓
Chemotherapy
↓
Egg reserve reduced to that of 40-year-old
↓
5-year delay for tamoxifen treatment
↓
Egg reserve reduced to that of 45-year-old
↓
Pregnancy probability nearly 0%

Breast Cancer Patients Have Sufficient Time for IVF Prior to Chemotherapy

- 6-week hiatus between surgery and chemotherapy
- Conventional stimulation raises estrogen
- Estrogen stimulates malignant cells[1,2]
- Natural cycle IVF results in single embryo[3]

1. Allred CD et al. *Carcinogenesis.* 2001;22:1667-1673.
2. Prest SJ et al. *FASEB.* 2002;16:592-594.
3. Omland AK et al. *Hum Reprod.* 2001;16:2587-2592.

Tamoxifen Was Originally a "Fertility Drug"

- Estrogen-receptor antagonist
- Invented in the United Kingdom as a post-coital contraceptive *(Harper. Nature, 1966)*
- Found to induce ovulation *(Klopper, BMJ, 1971)*
- Suppressed rat mammary cancer *(Jordan, Eur J Cancer, 1976)*

Letrozole for Ovulation Induction

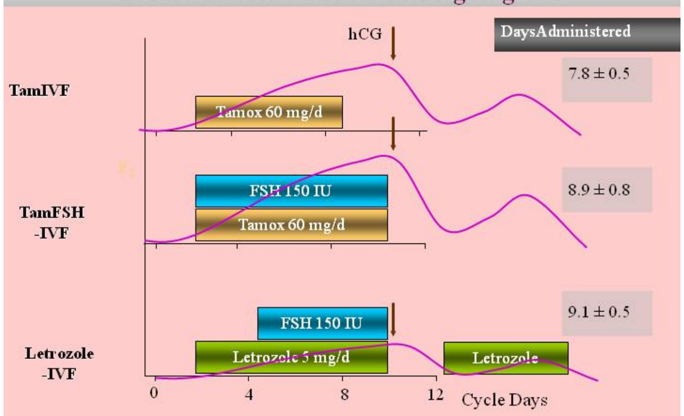
- Highly selective, third generation aromatase inhibitor (AI)
- Suppresses estradiol by up to 90%
- Alternative to tamoxifen in breast cancer[1]
- Used for ovulation induction recently
- 5 mg more effective than 2.5 mg/day dose[2]

[1] *Goss et al. NEJM 2003;349:1793-802*
[2] *Marinko M et al· Fertil. Steril. (2002) 78(Supplement 1):S55*

Design

- Institutional Review Board (IRB)-approved
- Prospective-controlled
- 2000-2005
- 86 breast cancer patients
- Stage I-III
- Desire fertility preservation

Prospective Comparison of Tamoxifen vs. Letrozole in Breast Cancer Patients Undergoing IVF



Oktay et al, J Clin Oncol 2005 Jul 1;23(19):4347-53.

Comparison of Cycle Characteristics and Outcome among Tam-IVF, TamFSH-IVF, and Letrozole FSH-IVF Patients

Variable	Tam-IVF	TamFSH-IVF	LetrozoleFSH-IVF	P value
Age	36.6 ± 1.6	38.3 ± 1.9	36.2 ± 0.8	NS
Baseline FSH (mIU/mL)	9.4 ± 1.5	9.4 ± 1.5	7.2 ± 0.8	NS
Peak E_2* (pg/mL)	419 ± 39[a,b]	1182 ± 271[a]	405 ± 45[a,b]	a < 0.01 b > 0.05
Total follicle	2 ± 0.3[a]	6 ± 1 [a,b]	8.3 ± 0.6[a,b]	a < 0.001 b > 0.05
Follicle ≥17mm	1.2 ± 0.1[a]	2.6 ± 0.4[a,b]	3.6 ± 0.3[a,b]	a < 0.05 b > 0.05
Total oocyte	1.7 ± 0.3[a]	6.9 ± 1.1[a,b]	11.0 ± 1.2[a,b]	a < 0.001 b > 0.05
Mature oocyte	1.5 ± 0.3[a,c]	5.1 ± 1.1[a,b,c]	8.0 ± 0.9[a,b]	a < 0.001 b,c < 0.05
Total 2-pro-nuclear embryo	1.3 ± 0.2[a]	3.8 ± 0.8[a,b]	5.3 ± 0.6[a,b]	a < 0.001 b > 0.05

Is Letrozole-IVF as Efficient as the Long Protocol?

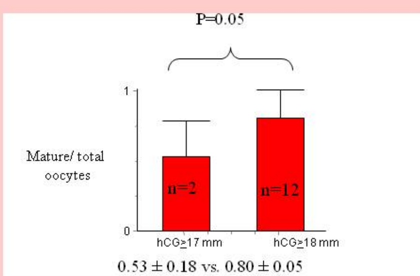
Table-2. Comparison of various characteristics between letrozole+FSH and control groups.

	Letrozole+FSH[a] mean ± standard error	Control[b] mean ± standard error	P-value[c]
Age at IVF	36.1 ± 0.5	36.9 ± 0.5	0.69
Baseline FSH	7.6 ± 0.5	4.3 ± 0.2	<0.001
Estradiol at hCG	459.1 ± 42	1453.3 ± 80.7	<0.001
Endometrial thickness	8.7 ± 0.4	10.8 ± 0.3	<0.001
Follicle N>17	4.0±0.2	2.6±0.2	<0.001
Peak follicle size (mm)	21.4 ± 0.4	18.8±0.2	<0.001
Total oocyte N	11.8±1	10.7±0.7	0.31
Mature oocyte N	8.4±0.7	9.2± 0.6	0.47
Mature oocyte (%)	74.3±3.4	84.3±1.9	<0.01
N of 2 pn embryos	6.3±0.6	6.6±0.5	0.65
Fertilization rate (%)	76.3.1±3.4	72.7±3.0	0.71
N of days stimulated	11.8 ±0.3	12.1±0.2	0.66
Total FSH dose (I)	1461.11±100	2355.0± 135.5	<0.001

a: 47 patients, 53 initiated IVF cycles resulting in 46 retrievals
b: 56 patients with male factor infertility, 64 initiated IVF cycles resulting in 63 retrievals
c: computed from the ANOVA test.

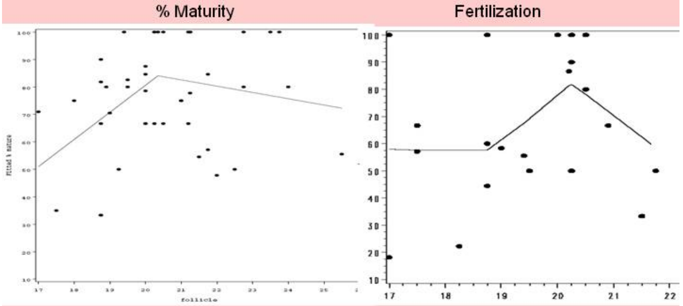
Oktay et al, JCEM 2006

Delaying hCG Injection Improves Mature Oocyte Yield in Let-FSH Stimulation



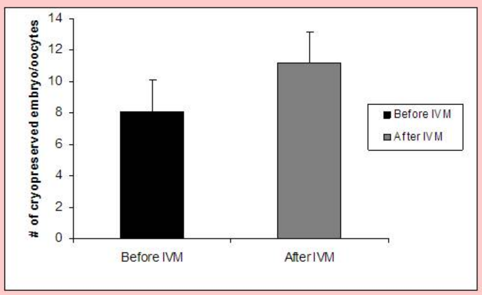
Let = letrozole; FSH = follicle-stimulating hormone; hCG = human chorionic gonadotropin

Peak Maturity and Fertilization When hCG is Given at 20 mm



Oktay et al, JCEM 2006

Utilization of IVM Further Increases the Yield of Oocytes and Embryos in Letrozole Cycles

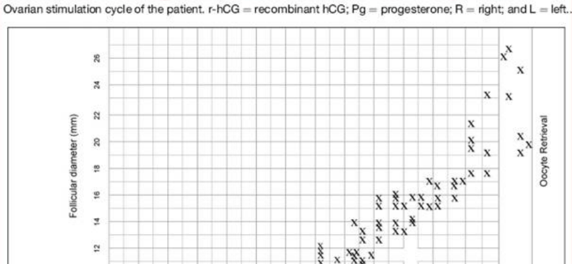


Embryo or oocyte yield increase 44.7±11.2 % (p<0.0001)

Oktay et al ASRM 2006

Oocyte Retrieval After Premature Surge During a Letrozole-IVF Cycle

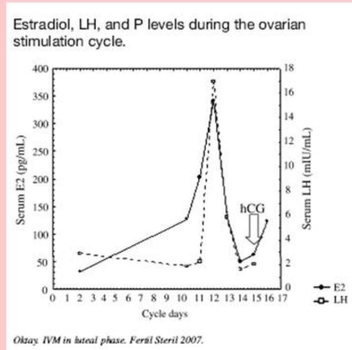
Ovarian stimulation cycle of the patient. r-hCG = recombinant hCG; Pg = progesterone; R = right; and L = left..

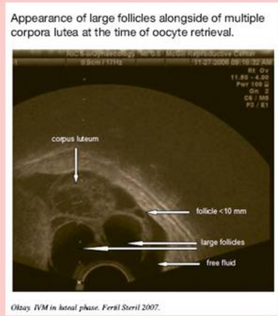


Ovary	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	L R	
Cycle day	1	2	5	6	7	8	9	10	11	12	13	14	15	16	17
Cetrorelix dose (mg)		3													
Letrozole dose (mg)				5	5	5	5	5	5	5	5	5			
FSH dose (IU)						300	300	300	300	225	300	300			
Ganirelix dose (mg)			0.25								0.25	0.25			
r-hCG dose (µg)													250		
E2 serum level (pg/mL)		32.7						127	203	342	129	50.4	63.2	125	
LH serum level (mIU/mL)		2.94						1.86	2.22	16.9	5.88	1.59	2.05		
Pg serum level (ng/mL)												5.69	8.38		

Oktay. IVM in luteal phase. Fertil Steril 2007.

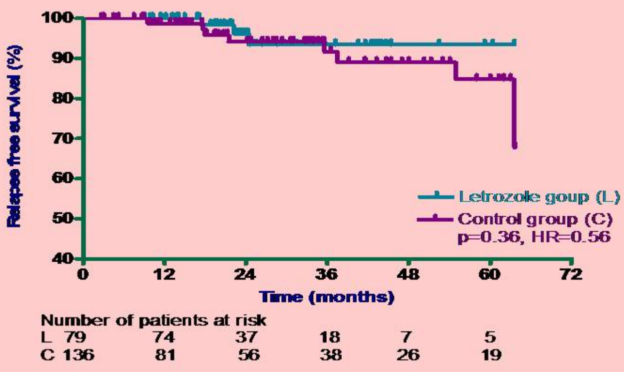
IVM with Oocytes Retrieved After Premature Luteinizing Hormone (LH) Surge

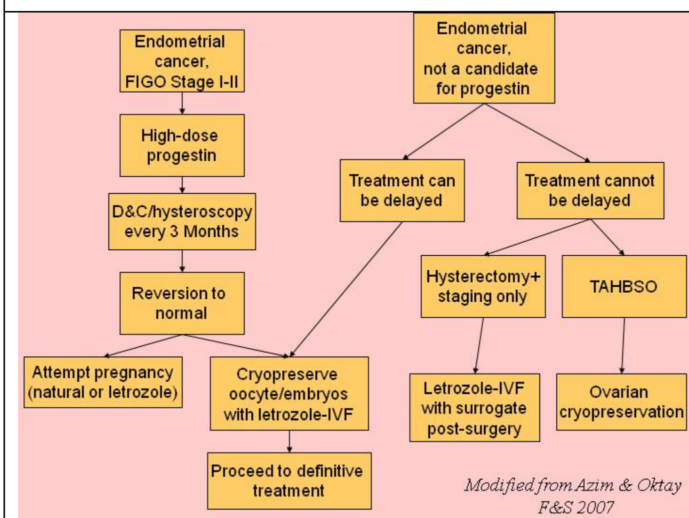




•2/4 with IVM and frozen at 4-cell stage on day 2

Impact of Letrozole-IVF on Relapse-Free Survival





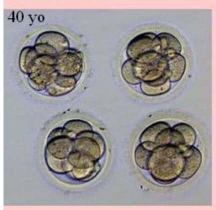
Letrozole for Endometrial Cancer Patients

	Protocol	E2 on hCG day (pg/ml)	Length of COH (d)	Oocytes retrieved	Mature Oocytes	Oocytes fertilized	Embryos frozen
A I	Letrozole 5mg x5 d	118	7	2	2	2	2
II	Letrozole FSH 150U, Ganirelix	353	14	Cancelled, premature ovulation	-	-	-
III	Letrozole FSH 225 hMG 225 U, Ganirelix	774	18	19	13	10	0
B	Letrozole FSH 112U	241	10	10	9	8	8
C	Letrozole FSH 150U	228	12	4	3*	1	1

*After in Vitro maturation
COH; controlled ovarian hyperstimulation, CD; cesarean delivery.

Azim & Oktay F&S 2007

Normal Embryo Development After Letrozole-FSH Stimulation



High Pregnancy Rates with Letrozole-IVF

Age at IVF cycle (y)	38.57 ± 3.13	37.63 ± 3.62	0.54
Day 2 FSH (mIU/mL)	7.7 ± 2.48	9.34 ± 4.83	0.17
Day 2 Estradiol (pg/mL)	35.06 ± 11.67	42.5 ± 20.54	0.7
Table-1	Pre-treatment group(n=14)	Post-treatment group (n=8)	P
Delivery/ongoing rate per transfer	8/18 (44.4)	3/15 (20)	0.48
Pregnancies	13	4	
Deliveries	7	2	
Ongoing	1	1	

Live birth rate is 37% for the same age group with standard IVF

Azim& Oktay, ASRM 2007

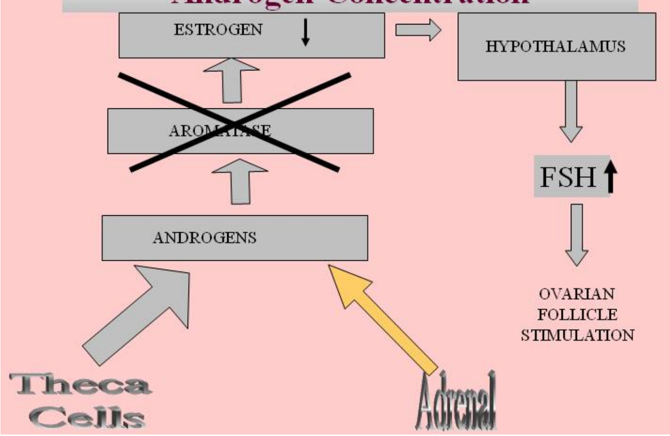
Higher Implantation Rates with Letrozole-IVF vs. Standard-IVF?

	Group FSH + letrozole	FSH + no letrozole
No. of patients	10	10
Median age in years (SD)	29.9 (2.78)	32.7 (5.82)
Indications		
Andrological	8	7
Tubal	1	1
Unexplained	1	2
Mean no. of oocytes (SD)	13.8 (9.24)	9.6 (7.73)
Fertilization rate % (SD)	63.3 (19.61)	77.84 (18.36)
Mean no. of embryos transferred (SD)	1.60 (0.52)	1.60 (0.84)
Mean no. of embryos cryopreserved (SD)	2.9 (3.81)	2.55 (4.21)
Positive HCG rate per cycle %	50	20
Clinical pregnancy rate per cycle %	50	20
Implantation rate % (ratio)	31.25 (5/10)	12.5 (2/10)

The results in the two groups are not statistically different using the Wilcoxon signed rank test.
HCG = human chorionic gonadotrophin.

RBMOnline - Vol 13. No 2. 2006 166-172 Reproductive BioMedicine Online; www.rbmonline.com/Article/2261 on web 19 May 2006

Aromatase Inhibition Increases Local Androgen Concentration



Local Androgens May Promote Early Follicular Development

- Androgens serve as a substrate for E_2 production
- Promote the growth of small follicles
- Promote proliferation of granulosa and theca cells
- Stimulate FSHr, IGF-Ir, IGF-I

FSHr = FSH receptor; IGF = insulin growth factor

Weil SJ et al. JCEM 1998;83:2479-85
Hillier SG in Molecular biology of female reproductive system,1994:1-37.

High E_2 Narrows Implantation Window

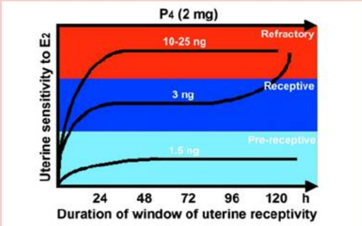


Fig. 3. A scheme depicting modulation of the window of receptivity in the P_4-primed uterus in response to changing estrogen levels. This scheme shows that estrogen at a low threshold level extends the window of uterine receptivity for implantation, but higher levels rapidly close this window, transforming the uterus into a refractory state.

Wen-ge Ma et al. PNAS 2003;100;2963-298

Safety of Letrozole?

- **4.7 vs. 1.8% major anomalies**
- **150 letrozole babies vs. 36,000 babies in low-risk hospital**
- **Laryngomalacia, craniosynostosis, sacral fusion, aortic stenosis (2), cystic lymphangioma, hepatocellular cancer**
- **Only locomotor anomalies significantly increased**

ASRM 2005 abstract

Flaws of "Letrozole Abstract"

- **Inappropriate control group:**
  - **Infertile vs. general population**
  - **Mean age 35 years vs. 30 years in controls**
  - **16% vs <1% multiple births**
  - **Anomalies would have been referred to tertiary care centers in controls**
- **21 babies lost to follow-up in letrozole**

No Increase in Congenital Malformations with Letrozole Treatment

- 911 infants from letrozole or clomiphene citrate (CC)
- Similar rates of malformation:
  - 2.4% vs. 3.0% in CC
  - Cardiac anomalies LESS frequent in letrozole:
    - 0.2 vs. 1.8% (p=0.02)

Tulandi---Casper. Fertil Steril, June 2006, in press

Further Evidence on the Safety of Letrozole

- 117 children born after letrozole
- 161 conceived with clomiphene
- 2.56% vs. 3.10% major anomalies (p>0.05)

[1] Padte K. Padte JK, Gadkar J. Major congenital anomalies following conception with clomiphene versus letrozole. Proceedings of the 2nd Serono Symposia on Regulation of Follicle Development and its Clinical Implications. May12-13, 2006, Beaune, France. Abst P-7.

No Congenital Anomalies with Continuous Letrozole-IVF

GA (w)	Mode of delivery	Birth weight (kgm)	Apgar	Pregnancy complications	Neonatal Complications	Follow-up Length (months)
34	CS	2.7, 2.54	7/8	PTL,Twins		1
39	VD	2.95	9/9	None		8
31	CS	1.54, 1.54, 1.33	7/8	PTL,PIH,Triplet	RDS,NICU	8
34	VD	1.88	7/8	PTL	NICU	15
39	VD	3.31	9/10	Ist trimester bleeding		4
36	VD	3.4	7/8	PROM	RDS, Clavicle Fracture	17
39	CS	3.69	8/9	Malpresentation		24
34	CS	2.0	8/9	PIH		23
39	CS	3.24	9/9	Placenta Previa		6

Azim & Oktay, ASRM abstract 2007

Lack of Biological Plausibility

- Letrozole has a 48-hour half-life.
  - Drug is cleared before fertilization in 5-day protocol.
- Cryopreserved embryo is never exposed.
- No evidence of effect on oocytes/embryos in mice.

R. Luthra et al. / Journal of Steroid Biochemistry & Molecular Biology 86 (2003) 461–467

Hu et al. Mol Reprod Developm 2002; 61:549-559

Lack of Birth Defects after Letrozole Treatment

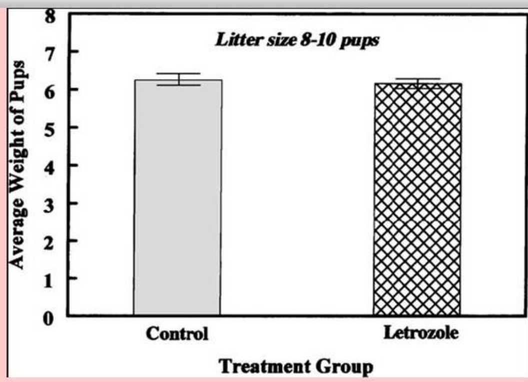
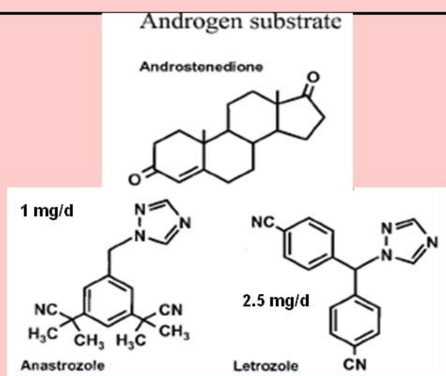


Fig. 1. Effect of letrozole treatment prior to pregnancy on birth weightand litter size of aromatase transgenic mice. Animals were treated for 6 weeks at the age of 12–16 weeks with 0.5 g letrozole per mouse per day. After 2 weeks of resting period, the animals were paired with males. Litter size was noted immediately after the birth and weight of the pups was determined on day ten. Average data ± S.D. was used for graphical representation.

R. Luthra et al. / Journal of Steroid Biochemistry & Molecular Biology 86 (2003) 461–467

Anastrozole and Letrozole: Third Generation Aromatase Inhibitors



Lack of Spindle Defects after In Vitro Exposure of Mouse Follicles to Anastrozole

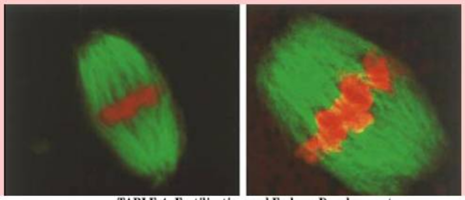


TABLE 4. Fertilization and Embryo Development

	No. of oocytes for fertilization	% of oocytes with PB/ total oocytes	% of 2-cell embryos/PB oocytes	% of blastocysts/2-cell embryos
In vivo controls	179	100	76	81
In vitro controls	108	62[a]	64[c]	74
Arimidex 50 μM	116	83[a,b]	45[a,d]	84

Values with different superscripts within a column are significantly different: [a]vs. in vivo controls ($P < 0.001$); [b]vs. in vitro controls ($P < 0.01$); [c]vs. in vivo controls ($P < 0.05$); [d]vs. in vitro controls ($P < 0.01$).

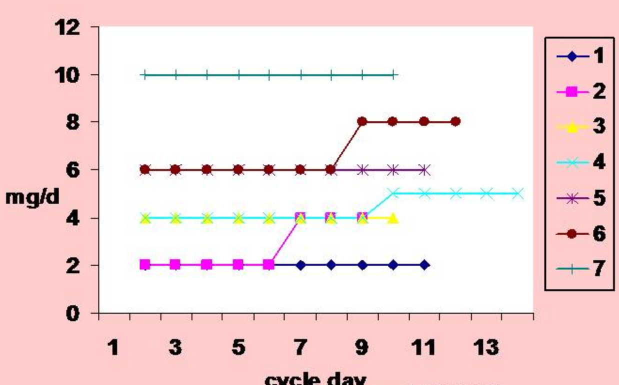
Hu et al. Mol Reprod Developm 2002; 61:549-559

Anastrozole vs. Letrozole in Breast Cancer

	Anastrozole (n=7)	Letrozole (n=47)	*p*
Age	36.2±1.4	36.37±0.5	NS
Day 2 FSH	10.27±1.12	7.41±0.54	NS
Length of stimulation	10.7±2.06	9.83±2.4	NS
Total gonadotropin dose	1854.36±526.69	1469.23±741	NS
E_2 on hCG day	**1325.89±277.72**	**427.78±42.42**	**<0.01**
E_2 on day following hCG	**2515.07±558.69**	**714.38±69.7**	**=0.01**
Number of follicles >17mm	2.67±1.58	3.84±1.72	NS
Number of oocytes retreived	9.71±3.21	11.57±1.0	NS
Number of mature oocytes	5.57±1.49	8.33±0.76	NS
Oocytes fertilized	3.57±1.07	6.17±0.62	NS
Embryos cryopreserved	5.57±1.84	6.20±0.63	NS

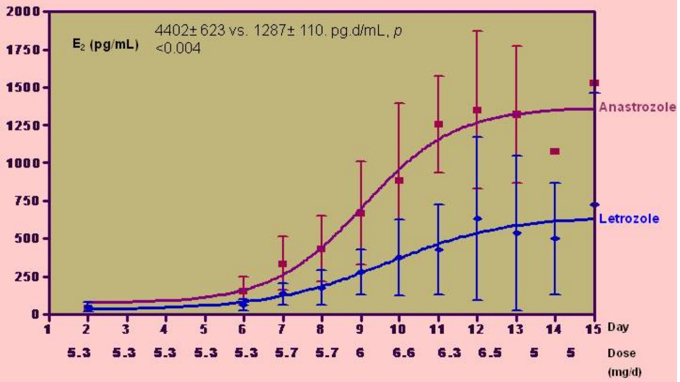
Azim et al, JCEM, 2007

Daily Anastrozole Dosing



Azim et al, JCEM 2007

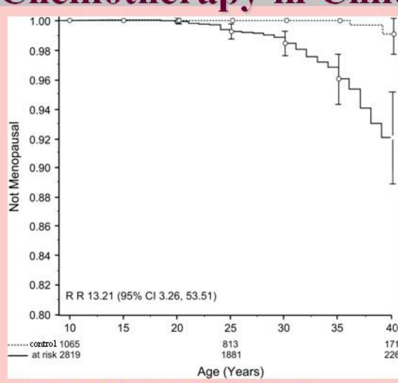
Average Daily Dose, Mean E_2 and Area Under the Curve $E_2(AUC-_{E2})$



Fertility Preservation in Children


Daily Mail
Agony of the bears they kill for fun
DIANA LANDS IN POLITICAL ROW
Harriet's hope for the future
Don't bank on cheaper loans

Increased Risk of Ovarian Failure After Chemotherapy in Childhood



Sklar, C. A. et al. J. Natl. Cancer Inst. 2006 98:890-896

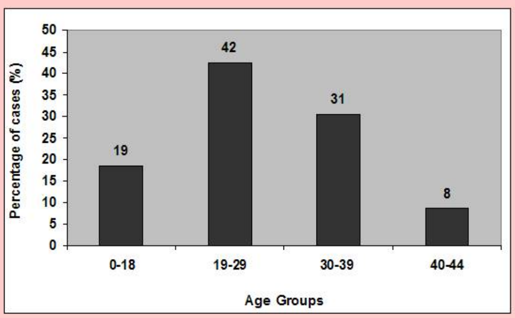
JNCI Journal of the National Cancer Institute

Stem Cell Transplantation Risks for Fertility

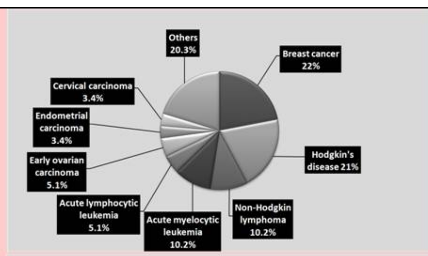
- High dose chemotherapy with or without total body irradiation (TBI)
- >95% ovarian failure and infertility
- Increased risk of pregnancy loss and preterm labor if received TBI

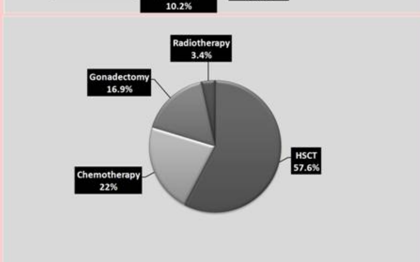
Sanders JE et al, Blood 1996;87:3045-3052.

Age Distribution of Patients Undergoing Ovarian Tissue Freezing



* $p<0.05$





Fertility Preservation in Impending POI (POF)

- 15-year-old with primary amenorrhea
- Otherwise normal sexual development
- Tanner stage IV
- In February 2008:
  - FSH: 92 mIU/mL
  - LH:25 mIU/mL
  - E_2: 23 pg/mL

POI = premature ovarian insufficiency
POF = premature ovarian failure

Clinical Course

- Repeat labs 4 weeks later:
  - FSH down to 11 mIU/mL, (Elevated for age ; Barad D et al. Gynecol. 2007 Jun;109(6):1404-10.)
  - E_2: 29 pg/mL
  - LH: 29 mIU/mL
- Referred by pediatric endocrinologist for fertility preservation

Pre-Fertility Preservation Evaluation

- On March 31, 2008, at CHR:
  - US: AFI = 5; endometrial thickness = 6 mm
  - FSH: 13.2 mIU/mL
  - E_2: 41 pg/mL
  - LH: 7.4 mIU/mL
  - AMH: 2.2 ng/mL

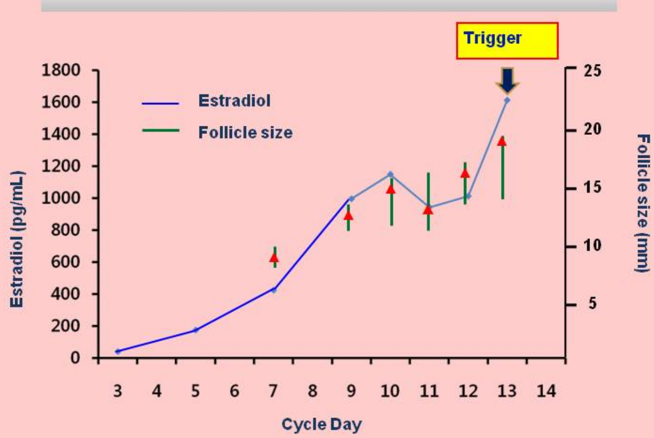
CHR = Center for Human Reproduction
US = ultrasound
AFI = amniotic fluid index
ET = endometrial thickness

Ovarian Stimulation

- No attempt to induce bleed
- Started with 300 IU recombinant FSH
- Step-down protocol with antagonist
- Transabdominal US monitoring
- 11 days of stimulation

Cycle Day	3	4	5	6	7	8	9	10	11	12	13	14	15
r-FSH HMG	300	300	225	225	150	150	137.5	150	150 75	150 150	Trigger	Retrieval	
Antagonist							1	1	1	1			
E_2 (pg/mL)	41		176		425		996	1150	944	1013	1613		
FSH (IU/L)	13.2		17		17		15.8	11.2	11.3	12.0	16.7		
LH (IU/L)	7.4		1.9		1.1		3.4	0.7	< 0.2	< 0.2	0.5		
Rt. Ovary	AFC 3				9.5 8.7 8.6		13	16 15.5 15.5 15 12.5	14.5 13.5 12	17 16.5 15.5 15	16 15.5 14.5		
Lt. Ovary	AFC 2				10 9 8.5 8.2 5		14 14 13.5 11	14.5 12.5 12	17.0 13.5 13 12.5 11.5	17.5 16.5 15.5 15 14	19.5 19.5 19.5 19 17.5		

Ovarian Stimulation in POF



Oocyte Freezing in Impending POI: the Day of Egg Retrieval

22 oocytes retrieved

Turner's Syndrome and Fertility

- 3-5% pregnancy (mostly mosaics)
- 29-66% pregnancy loss
- High miscarriage rate (40%), even with donor egg
- Primordial follicles found up to age 12-13

Hreinsson et al, JCEM 2002, 87:3618-23

Fertility Preservation in Turner's Syndrome

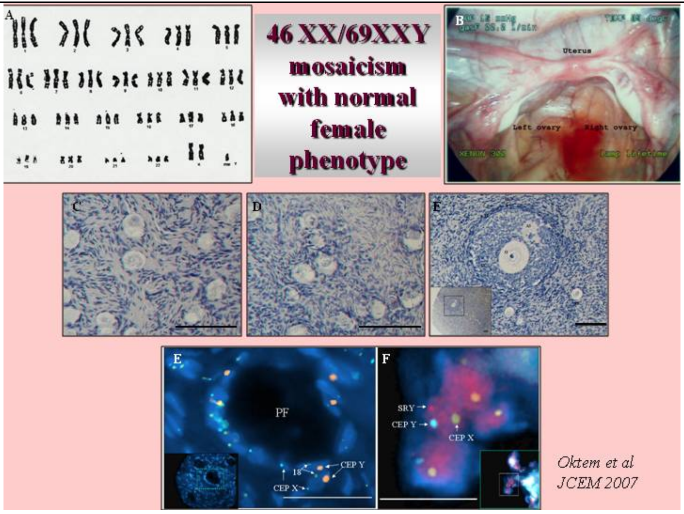
14-year-old female with impending POF-mosaic Turner's syndrome

↓

Ovarian stimulation

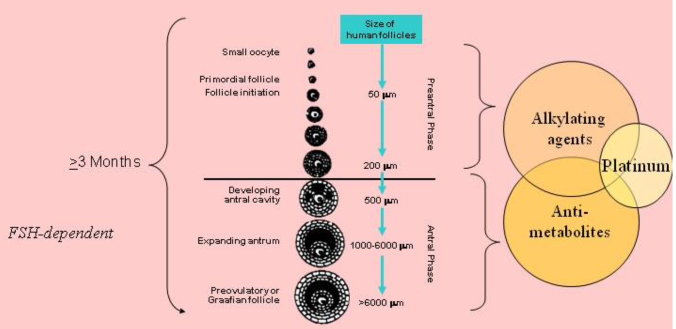
↓

11 oocytes cryopreserved



Feasibility of Ovulation Induction During or Immediately After Chemotherapy

Different Classes of Agents Affect Different Stages of Follicular Growth



Concerns with Ovarian Stimulation During or Immediately After Chemotherapy

- Residual DNA damage:
  - Mutations, breakage, aneuploidy in somatic cells
- Oocytes typically not viable/poor response
  (Pdydn, E. and Ataya, K. (1991) Rep. Toxicol., **5***, 73–78)*
- Increased anomalies and pregnancy losses
  (Meirow D et al, Hum Reprod 2001)

Can Ovarian Stimulation Be Performed Immediately After Initiation of Chemotherapy?

- Female mice superovulated
- Cytoxan 0-24 days before oocyte recovery
- Significantly reduced in vitro fertilization and cleavage rates
- Improvement when oocytes recovered >3 days after chemotherapy

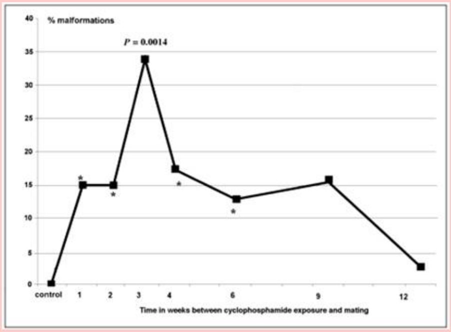
Pdydn, E. and Ataya, K. (1991) *Rep. Toxicol.*, **5**, 73–78.

Rodent Studies Show High Pregnancy Failure and Malformation

- Mating 1-4 weeks after cyclophosphamide (75 mg/kg)
- High rate of implantation failure
- 10X malformation rate
- Risk normalized when mating delayed to 12 weeks post-chemotherapy.

Meirow D et al. Hum Reprod 2001

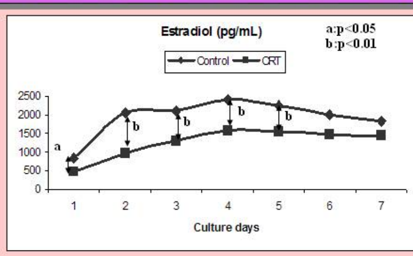
Malformation in Pregnancy Immediately Following Chemotherapy



Meirow D et al. Hum Reprod 2001

IVF Post-chemotherapy

Decreased estradiol (E_2) Secretion in Vitro of Ovarian Pieces from Women Previously Exposed to Chemo- and or Radiotherapy (CRT)



	Control	CRT	p value
Mean age	27.7 ± 2.4	27.5 ± 2.7	>0.05
Follicle density PF/mm²	9.31 ± 2.6	5.78 ± 1.5	<0.05
Mean Estradiol	1891.8 ± 192.6	1141.7 ± 116.6	<0.05

Oktem and Oktay, Cancer 2007

IVF Outcome Post-chemotherapy in Women with Normal Day-2 FSH and E_2

	Post-chemo (n=22)	Pre-chemo or radiation (n=43)
Mean Age	36.5±0.8	35.7 ± 0.9
Total cycles	38	76
Cycles with retrieval (%)	28 (73.7)	68 (89.4%)
Cycles with ET (%)	17 (44.7)	8 (10.5%)
Oocytes retrieved (%)*	4.6 ± 0.9	12.4 ± 7.0
Mature oocytes*	3.7 ± 0.8	8.7 ± 4.8
Oocytes fertilized*	2.9 ± 0.7	6.6 ± 4
Clinical pregnancy/ET	5/17 (29%)	5/8 (62.5%)
Live /cycle (%) **	3/17 (17.6%)	4/8 (50%)
Day 2 AMH (ng/mL)*†	0.27 ± 0.08	0.84 ± 0.3

Compiled from Oktay et al, JCEM 2006 and Azim & Oktay, ASRM abstract, 2006 — **self for gestational carrier*

Summary

- Ovarian stimulation in cancer patients is more complicated due to medical issues.
- Letrozole + FSH may be safe and effective in patients with estrogen-sensitive cancer.
- Post-chemotherapy response is poor.

Summary

- Letrozole-FSH and tamoxifen-FSH protocols maybe safe and effective for breast cancer patients.
- Letrozole can be used to stimulate endometrial cancer patients.

Summary

- In prepubertal children, the only available fertility preservation technique is tissue freezing.
- In relatively mature post-pubertal girls, oocyte cryopreservation maybe utilized.
- All options discussed in this lecture are experimental.

Announcements

- American Society for Clinical Oncology (ASCO) Clinical Guidelines for Fertility Preservation
- ASRM Fertility Preservation Special Interest Group
- Fertile Hope: www.fertilehope.org
- www.fertilitypreservation.org

Novartis Disclaimer

It is "aware that Femara is being used to stimulate ovulation in women who are infertile, or unable to become pregnant, as a treatment to increase their chances of becoming pregnant."
The drug "should not be used in women who may become pregnant, during pregnancy and/or while breast-feeding, because there is a potential risk of harm to the mother and the fetus, including risk of fetal malformations," the company said.

Sharks on the Hunt!

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NOTES

NOTES

OTHER OPTIONS: IVM AND GONADAL SUPPRESSION

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LEARNING OBJECTIVES

At the conclusion of this presentation, participants should be able to:

1. Discuss the process of in vitro maturation (IVM).
2. Explain the use of gonadotropin-releasing hormone (GnRH) agonists.
3. Determine when IVM can be used.

Other Options: In Vitro Maturation (IVM) and Gonadal Suppression

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Learning Objectives

At the conclusion of this presentation participants should be able to:

1. Discuss the process of in vitro maturation (IVM).
2. Explain the data on the use of gonadotropin-releasing hormone (GnRH) agonists.
3. Determine when IVM can be used.

Disclosure

Lynn M. Westphal
Advisory Board:
EMD Serono, Schering Plough, Ferring

Options for Fertility Preservation

- Cryopreservation of oocytes
- Cryopreservation of embryos
- Cryopreservation of ovarian tissue
- Co-treatment with GnRH agonists
- Chemotherapy with less ovarian toxicity
- Oophoropexy
- Fertility-sparing surgery

Endocrine Protection

Montz et al. 1991

Group	% fertile	Litter size
Control	93	13.3
CTX	80	6.3
CTX+L 24 hours	71	0.7
CTX+L 12 hours	100	8.0
CTX+P	100	11.4

CTX = chemotherapy
L = leuprolide
P = progesterone

Hormonal Suppression

- Non-cycling cells are more resistant to damage from antineoplastic agents.
- In men, it is proposed that interruption of the pituitary-gonadal axis would rest testis and protect it.
- However, most studies have shown no benefit in men.

Hormonal Suppression

- Only one randomized study of effect of GnRH agonist (Waxman, 1987); small study with 18 patients showed not benefit.
- Need results from ongoing prospective trials

Ovarian Suppression

Blumenfeld, 2008

- Studied women (age 15-40 years) receiving chemotherapy for Hodgkin's disease (not randomized)
- 63 of 65 (96.9%) women treated with GnRH agonist resumed menses (POF-both age 36).
- Only 29/46 (63%) of control patients resumed menses.

POF = premature ovarian failure

Ovarian Suppression

Blumenfeld, 2008

- Did not show difference in pregnancy rates, although many had not attempted pregnancy
- No significant difference in ABVD group (only 1 of 10 in control with POF)
- Control group: BEACOPP 36% POF; MOPP/ABVD 50% POF

ABVD = doxorubicin, bleomycin, vinblastine and darcarbacine
BEACOPP = bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, procarbazine, and prednisone
MOPP = mechlorethamine, vincristine, procarbazine, and prednisone

Proposed Mechanisms

Blumenfeld , 2008

- Prevents increase in follicle-stimulating hormon (FSH)
- Decreases utero-ovarian perfusion
- Direct effect on ovary
- Up-regulates intragonadal antiapoptotic molecule
- Protects undifferentiated germline stem cells

Ovarian Protection in Breast Cancer

Recchia 2006

- Studied 100 premenopausal women with breast cancer (ages 27-50 years, median age 43 years)
- All patients received GnRH agonist for one year with adjuvant chemotherapy
- All patients under age 40 resumed menses
- 56% of patients > 40 years resumed menses
- Good disease-free survival; perhaps clinical benefit for estrogen receptor positive (ER+) cancer

Ovarian Suppression with Agonist/Antagonist

Potolog-Nahari , 2007

- 9 patients (mean age 26.6 years)
- Given GnRH agonist and antagonist simultaneously
- 8/9 (88.9%) had normal hormone levels in 3-6 months and resumption of menses in 3-11 months

Prevention of Menorrhagia

Meirow , 2006

- Incidence of moderate/severe menorrhagia in women with severe thrombocytopenia (25,000) after chemotherapy
- None of patients receiving GnRH agonist
- 9/42 (21.4%) of women receiving DMPA
- 9/30 (40%) of women untreated

DMPA = depot medroxyprogesterone acetate

Apoptotic Inhibitors

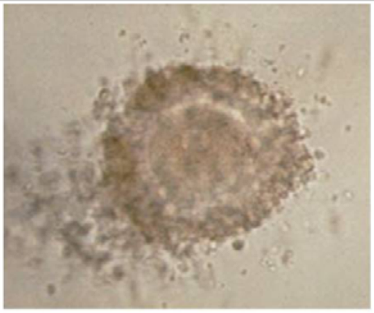
Morita , 2000

- Apoptosis is a mechanism for germ cell loss
- Ceramide is a sphingolipid molecule that may be an early signal for apoptosis
- Mice that received sphingomyosine-1-phosphate therapy (to counteract ceramide), resisted oocyte apoptosis induced by radiation

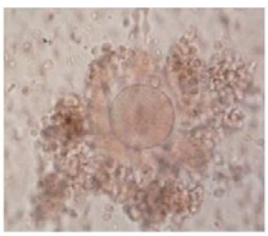
In Vitro Maturation (IVM)

- World's first IVF baby was from an unstimulated cycle.
- Conventional IVF: ovarian stimulation used to increase the number of eggs.
- In IVM, patients receive no/minimal stimulation medications.
- Veeck reports pregnancy from in vitro matured oocytes in 1983.
- In 1994, Trounson reported first birth from IVM in polycystic ovary syndrome (PCOS).
- After egg retrieval, immature eggs are cultured in the lab for 24-48 hours.
- Mature eggs are then fertilized with intracytoplasmic sperm injection (ICSI) technique.

Immature Oocyte



36 Hours Later



In Vitro Maturation

- Best candidates are women under age 40 years who have multiple small follicles (PCOS).
- These women are at higher risk of over-responding to fertility medications and developing complications.
- In vitro maturation (IVM) may be a safer way to treat these patients.

Benefits of IVM

- No or minimal doses of gonadotropins
- Easier schedule/less monitoring
- Decreased cost of medications
- Decreased risk of complications related to medications, such as ovarian hyperstimulation syndrome (OHSS)

Follicular Priming

- Should patients receive some stimulation?
  - Follicle-stimulating hormone (FSH) early in the cycle
  - Human chorionic gonadotropin (hCG) before retrieval
- Or no medication at all?

Follicle Priming

- Several studies show that IVM can be successful without any ovarian hyperstimulation.
- Some studies have shown evidence that IVM embryos from unstimulated cycles show suboptimal development and decreased implantation and pregnancy rate (PR).

Follicle Priming

- PCOS patients may benefit from follicle priming.
- Suikkari et al. 2000, compared patients with regular cycles without stimulation and PCOS patients with stimulation and found similar outcomes.
- Mikkelsen et al. 2001, randomized PCOS patients to stimulation (FSH 150 IU for 3 days) vs. no stimulation, and found 29% PR in stimulated patients vs. no pregnancies in unstimulated patients.

HCG Priming?

Chian RC, 2000

	+hCG (n=13)	-hCG (n=11)
Age	35.3	34.5
# eggs	7.8	7.4
% Matured eggs (48hrs)	84.3%	69.1%*
% fertilization	90.7	83.9
Pregnancy rate	38.5%	27.3%*
# transferred	2.8	2.5

* = statistically significant

When To Retrieve?

Son WY, 2008

- 3 groups: leading follicle <10 mm, 10-14 mm, >14mm
- Retrieval after hCG priming
- Rates of IVM, fertilization, embryo development comparable
- Clinical pregnancy rate highest in 10-14 mm group

Oocyte Retrieval: Technique

- Compared to IVF:
  - Needle is more rigid and shorter (to accumulate less aspirate volume).
  - Bevel length of needle is shorter (in order to fit into the smaller follicles).
  - Lower aspiration pressure of 50-80 mm Hg vs. 80-100 mm Hg in IVF
  - Technique is different and takes longer
    - Collapse of follicle not readily seen
    - Requires multiple passes through the ovary
    - May flush with solution that contains heparin

Identifying Oocytes

- Immature oocytes are smaller and more difficult to identify
  - Requires more training/practice
  - Can have mature oocytes from follicles as small as 8-10 mm

IVM Safety

- Estimated that 400 children have been born through IVM cycles
- No known increase in birth defects
- Buckett et al. 2007, reported no difference between 55 IVM children, 217 IVF children, and 160 ICSI children born in the same time period (1998-2003).

IVM Protocol

- Baseline ultrasound cycle day (CD) 2-3
- FSH 150 IU CD 3-5
- Ultrasound every 1-2 days starting CD 6
- Retrieval when lead follicle is 10-14 mm and endometrium > 5mm
- hCG 35 hours before retrieval

IVM Protocol

- Evaluate oocyte maturation 1 and 2 days later
- ICSI when oocytes mature

IVF vs. ICSI

- ICSI is favored method
- Potential zona hardening from culture
- Cumulus stripped to identify oocyte maturity
- One semen collection adequate (over 48 hours)

IVM/Embryo Vitrification

Chian RC, 2001

- 31-year-old with PCOS
- Day 9 after withdrawal bleed, given hCG
- 63 oocytes obtained; 10 abnormal; 41/53 mature after 24-48 hours in maturation medium
- 31/41 fertilized with ICSI; 16 frozen at pronuclear stage
- Term delivery with frozen embryos

IVM/Oocyte Vitrification

Chian RC, 2009

- 27-year-old with PCOS
- Day 11: largest follicle 10 mm; given hCG
- Mature oocyte vitrified immediately; immature oocytes (18) cultured 24-48 hours in maturation medium
- 16 reached metaphase II (MII) stage and vitrified

IVM/Oocyte Vitrification
Chian RC, 2009

- Two months later: all thawed, 4 survived
- ICSI performed: 3 fertilized
- Term delivery of healthy infant

IVM after Premature LH surge
Oktay K, 2008

- 40-year-old with breast cancer
- On 7th day of stimulation, luteinizing hormone (LH) rose to 16.9 mIU/mL; GnRH antagonist started
- 3 days later, leading follicles 19-20 mm; progesterone=8.38 ng/mL
- Concern that follicles luteinized

IVM after Premature LH Surge
Oktay K, 2008

- hCG given with retrieval 35 hours later.
- At retrieval, free fluid and multiple corpora lutea.
- No oocytes retrieved from larger follicles
- 4 germinal vesicle-stage oocytes (GVs) retrieved and placed in IVM medium
- 2 matured and underwent ICSI; 2 embryos vitrified

IVM after Oophorectomy

Huang JYJ, 2007

- 43-year-old with borderline ovarian tumor
- Visible follicles on removed ovary aspirated.
- 4 immature oocytes retrieved and placed in IVM medium.
- 3 oocytes matured and were cryopreserved.

IVM Conclusions

- Advantages of less cost, less OHSS risk, more patient-friendly, perhaps easier scheduling
- More labor-intensive
- Pregnancy rates to date inferior to IVF.

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NOTES

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EMERGING TECHNOLOGIES FOR THE PRESERVATION OF FERTILITY IN FEMALE CANCER PATIENTS

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LEARNING OBJECTIVES

At the conclusion of this presentation, participants should be able to:

1. Describe basic mechanisms by which anti-cancer treatments damage the ovaries.
2. Explain the potential value of protecting the ovaries in situ with anti-apoptotic compounds.
3. Openly consider the possibility that regeneration of the ovarian reserve using stem cell-based technologies may one day be feasible.

Emerging Technologies for the Preservation of Fertility in Female Cancer Patients

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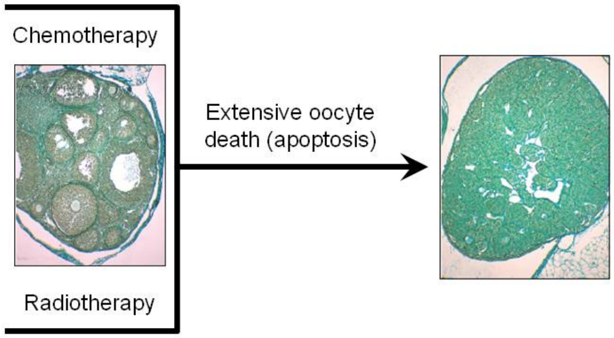
Disclosure

Jonathan L. Tilly, Ph.D.,

declares interest in the intellectual property associated with a patent describing the use of S1P as a therapeutic agent for the prevention of gonadal failure and the preservation of fertility (U.S. Patent Number 7,195,775).

Anti-Cancer Treatments and Ovarian Damage



Tilly, Nat Rev Mol Cell Biol 2001

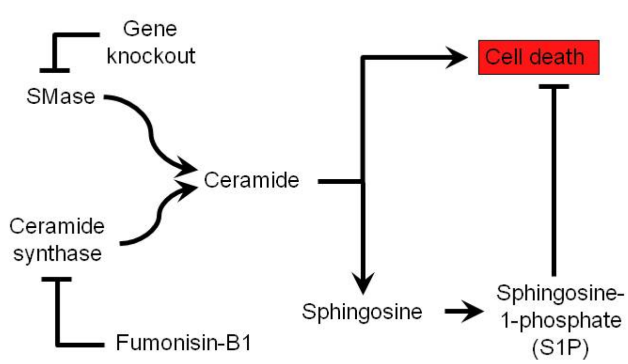
Strategies to Preserve Fertility and Ovarian Function

Prevent loss of the existing oocyte and follicle pool through anti-apoptotic small molecules

OR

Repopulate the ovaries with a new oocyte and follicle pool through stem cell-based regenerative medicine.

The Sphingomyelin Pathway and Cell Death



SMase =

In Rodent Models, S1P Has Been Shown To:

1. Prevent oocytes from undergoing apoptosis in response to chemotherapy exposure.
2. Protect ovaries in situ from damage caused by chemotherapy or radiotherapy.
3. Maintain fertile potential with birth of offspring that are free of anatomical and cytogenetic abnormalities.

Perez et al. Nat Med 1997
Morita et al. Nat Med 2000
Paris et al. Nat Med 2002
Jurisicova et al. Cell Death Differ 2006
Hancke et al. Fertil Steril 2007
Kaya et al. Fertil Steril 2008

A Critical Next Step in Validating S1P as a Fertility Preservation Agent

Preclinical testing of S1P and its mimetics as fertility preservation agents using female rhesus monkeys

Experiment 1 → Does intraovarian delivery of S1P protect oocytes from destruction?

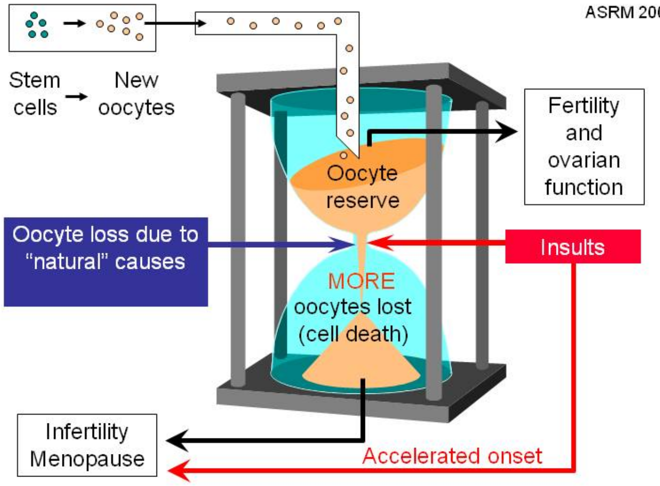
Experiment 2 → Does intraovarian delivery of S1P maintain female fertile potential: birth of healthy offspring?

Strategies To Preserve Fertility and Ovarian Function

Prevent loss of the <u>existing</u> oocyte and follicle pool through anti-apoptotic small molecules

OR

Repopulate the ovaries with a <u>new</u> oocyte and follicle pool through stem cell-based regenerative medicine.



For a detailed overview of the contemporary experimental evidence offered for and against the possibility that adult female mammals retain the ability to produce oocytes, see Tilly et al. Biol Reprod 2009

Johnson et al. Nature 2004

Postnatal mouse ovaries possess mitotically active germline cells that support oogenesis during adult life.

BIOLOGY OF REPRODUCTION 80, 2–12 (2009)
Published online before print 27 August 2008.
DOI 10.1095/biolreprod.108.069088

Minireview

The Current Status of Evidence for and Against Postnatal Oogenesis in Mammals: A Case of Ovarian Optimism Versus Pessimism?[1]

Jonathan L. Tilly,[2] Yuichi Niikura, and Bo R. Rueda

Mammalian Female Germline Stem Cells (FGSC)?

nature cell biology

Zou et al., Nat Cell Biol 2009

Production of offspring from a germline stem cell line derived from neonatal ovaries

Kang Zou[1], Zhe Yuan[1], Zhaojuan Yang[1], Huacheng Luo[1], Kejing Sun[1], Li Zhou[1], Jie Xiang[1], Lingjun Shi[1], Qingsheng Yu[1], Yong Zhang[1], Ruoyu Hou[1] & Ji Wu[1,2]

The idea that females of most mammalian species have lost the capacity for oocyte production at birth[1-5] has been challenged recently by the finding that juvenile and adult mouse ovaries possess mitotically active germ cells[6]. However, the existence of female germline stem cells (FGSCs) in postnatal mammalian ovaries still remains a controversial issue among reproductive biologists and stem cell researchers[6-10]. We have now established a neonatal mouse FGSC line, with normal karyotype and high telomerase activity, by immunomagnetic isolation and culture for more than 15 months. FGSCs from adult mice were isolated and cultured for more than 6 months. These FGSCs were infected with GFP virus and transplanted into ovaries of infertile mice. Transplanted cells underwent oogenesis and the mice produced offspring that had the *GFP* transgene. These findings contribute to basic research into oogenesis and stem cell self-renewal and open up new possibilities for use of FGSCs in biotechnology and medicine.

FGSC transplantation into chemo-sterilized females regenerates the ovarian reserve, leading to production of developmentally competent eggs

Evidence from Rodent Models that Stem Cell-based Regenerative Medicine Can Effectively Protect Adult Ovaries from Anti-Cancer Treatments

Bone marrow transplants regenerate the ovarian reserve (Johnson et al., Cell 2005) and restore long-term fertility (Lee et al., J Clin Oncol 2007) in chemo-sterilized mice.

Bone marrow mesenchymal stem cell transplants rescue ovarian function in chemotherapy-treated rats (Fu et al., Cytotherapy 2008).

FGSC transplantation into chemo-sterilized mice regenerates the ovarian reserve, leading to production of developmentally competent eggs (Zou et al., Nat Cell Biol 2009).

Is There Evidence form Clinical Observations that Stem Cell-based Regenerative Medicine Might Prove Useful for Fertility Preservation in Female Cancer Patients?

Unexpected spontaneous return of ovarian function and fertility in some patients receiving bone marrow transplants after high-dose chemotherapy (e.g., busulfan and/or cyclophosphamide) or radiation treatment

Samuelsson et al., Bone Marrow Transplant 1993
Salooja et al., Bone Marrow Transplant 1994
Sanders et al., Blood 1996
Salooja et al., Lancet 2001
Hershlag and Schuster, Fertil Steril 2002
Liu et al., Bone Marrow Transplant 2008

Isolation of Stem-like Cells with Germline Characteristics from Adult (Postmenopausal) Human Ovaries

Establishment and expansion in culture

Spontaneous generation of oocyte-like cells in vitro

Virant-Klun et al., Differentiation 2008

Oocyte-like cells can undergo parthenogenesis to form blastocyst-like structures in vitro.

Virant-Klun et al., Stem Cells Dev 2008

Summary of Key Points	
1. Anti-cancer treatments activate programmed cell death (apoptosis) in oocytes, which causes ovarian failure. 2. Small molecules that interfere with activation of apoptosis, such as sphingosine-1-phosphate (S1P), show significant promise for development as fertility preservation agents. 3. Adult mouse ovaries contain germline stem cells that generate new oocytes, and these oocytes yield viable offspring following fertilization. 4. Adult human ovaries contain stem-like cells with germline characteristics. 5. Stem cell transplants convey fertility-promoting effects in rodent models of chemotherapy-induced ovarian failure.	

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NOTES

AN INDIVIDUALIZED APPROACH TO FERTILITY PRESERVATION: MARRYING KNOWLEDGE OF REPRODUCTIVE BIOLOGY TO THE PRACTICE (CASE DISCUSSIONS)

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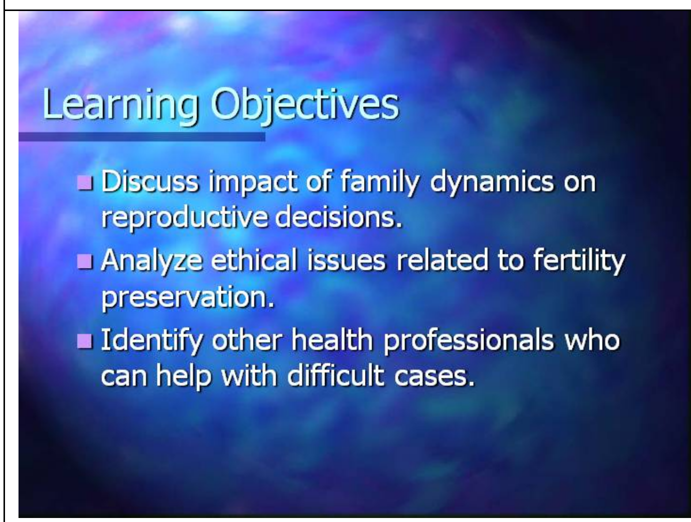
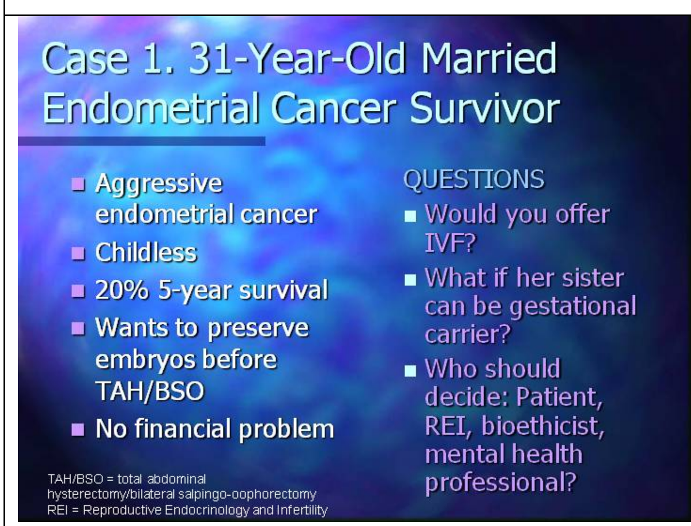
LEARNING OBJECTIVES

At the conclusion of this presentation, participants should be able to:

1. Discuss the impact of family dynamics on reproductive decisions.
2. Analyze ethical issues related to fertility preservation.
3. Identify other health professionals who can help with difficult cases.


Case Presentations
All Faculty


Learning Objectives
Discuss impact of family dynamics on reproductive decisions.
Analyze ethical issues related to fertility preservation.
Identify other health professionals who can help with difficult cases.


Case 1. 31-Year-Old Married Endometrial Cancer Survivor
Aggressive endometrial cancer
Childless
20% 5-year survival
Wants to preserve embryos before TAH/BSO
No financial problem
QUESTIONS
Would you offer IVF?
What if her sister can be gestational carrier?
Who should decide: Patient, REI, bioethicist, mental health professional?
TAH/BSO = total abdominal hysterectomy/bilateral salpingo-oophorectomy
REI = Reproductive Endocrinology and Infertility

Case 2. 4-Year-Old Girl with Wilm's Tumor

- To have abdominal radiation therapy with high risk for adult infertility
- Parents request cryopreservation pf ovary
- vs. ovarian transposition: Some successes but also adhesions and dyspareunia

QUESTIONS

- What are the pros and cons of ovarian cryopreservation?
- Who should make decision: Parents alone or with help from bioethicist?

Case 3. 35-Year-Old Single Woman Wants To Cryopreserve Oocytes

- Advertising executive has not met the right man to marry
- Wants to take precautions against reproductive aging

QUESTIONS

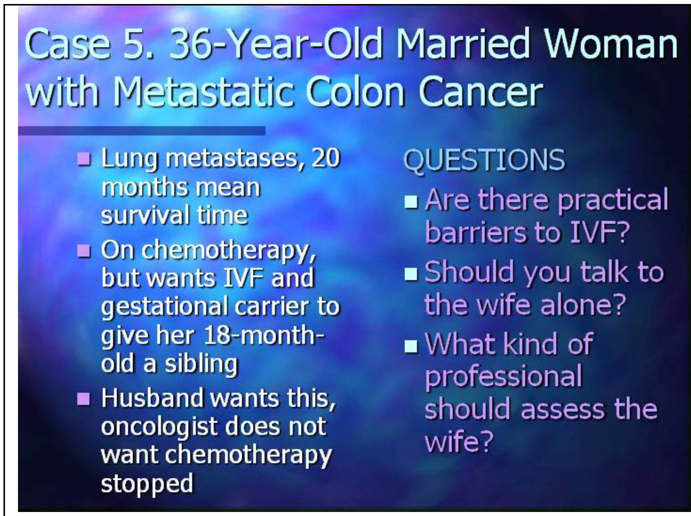
- Would you provide oocyte cryopreservation if feasible?
- If women are paying out-of-pocket, should society still ban this at least until consistent success rates?

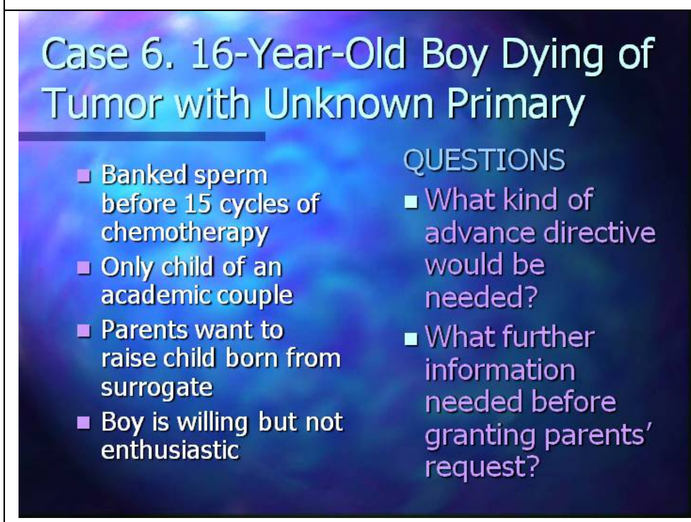
Case 4. 38-Year-Old Married Woman with Stage II Breast Cancer

- To have chemotherapy with high risk of infertility
- Wants IVF with letrozole and fresh embryo transfer to gestational carrier
- Couple does not want to wait because of their ages

QUESTIONS

- Would you go along with their wishes?
- If woman had BRCA2 mutation, would you make a different choice?


Case 5. 36-Year-Old Married Woman with Metastatic Colon Cancer
Lung metastases, 20 months mean survival time
On chemotherapy, but wants IVF and gestational carrier to give her 18-month-old a sibling
Husband wants this, oncologist does not want chemotherapy stopped
QUESTIONS
Are there practical barriers to IVF?
Should you talk to the wife alone?
What kind of professional should assess the wife?


Case 6. 16-Year-Old Boy Dying of Tumor with Unknown Primary
Banked sperm before 15 cycles of chemotherapy
Only child of an academic couple
Parents want to raise child born from surrogate
Boy is willing but not enthusiastic
QUESTIONS
What kind of advance directive would be needed?
What further information needed before granting parents' request?

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Course #5 Test Questions

1. In an experimental trial, metaphase II mouse oocytes are to be cryopreserved using different cooling and warming protocols after equilibration with 1.5 M dimethylsulfoxide at room temperature and aspiration into 0.25-mL plastic straws with a volume of 0.2 mL. In all cases, 1.5 M dimethylsulfoxide is to be removed by a stepwise dilution. Which one of the following protocols is more likely to give a better cryopreservation outcome?

    a. Cooling to -8ºC at 2ºC/minute, seeding extracellular ice and holding at -8 ºC for 10 minutes, cooling to -35ºC at 0.3ºC /minute, plunging into liquid nitrogen (LN_2); warming first in air for ~20 sec, and then in a water bath at 37ºC
    b. Cooling to -8ºC at 2ºC/minute, seeding extracellular ice and holding at -8ºC for 10 minutes, cooling to -35 ºC at 0.3 ºC /minute, plunging into LN_2; warming to room temperature at 5ºC/minute
    c. Cooling to -8ºC at 2ºC/minute and holding at -8ºC for 10 minutes, cooling to -35ºC at 0.3ºC /minute, plunging into LN_2; warming first in air for ~20 sec, and then in a water bath at 37ºC
    d. Rapid cooling to 0ºC and then cooling to -6ºC at 2ºC/minute, seeding extracellular ice and holding at -6ºC for 10 minutes, cooling to -35ºC at 0.3ºC /minute, plunging into LN_2; warming first in air for ~20 sec, and then in a water bath at 37ºC
    e. Plunging the samples into LN_2 and warming in a water bath at 37ºC

2. Which one of the following best describes critical improvements that led to successful cryopreservation of human oocytes after slow cooling and vitrification?

    a. Slow cooling: use of intracytoplasmic sperm injection (ICSI), addition of a sugar such as sucrose to cryopreservation solutions; vitrification: use of ICSI, increasing penetrating cryoprotectant (CPA) concentrations in vitrification solutions, further increasing the cooling and warming rates by minimizing the sample volume and using open carriers
    b. Slow cooling: use of ICSI, use of ethylene glycol as a penetrating CPA; vitrification: use of ICSI, increasing penetrating CPA concentrations in vitrification solutions, further increasing the cooling and warming rates by minimizing the sample volume and using open carriers
    c. Slow cooling: use of ICSI, addition of a sugar such as sucrose to cryopreservation solutions; vitrification: use of ICSI, decreasing penetrating CPA concentrations in vitrification solutions, further increasing the cooling and warming rates by minimizing the sample volume and using open carriers
    d. Slow cooling: use of ICSI, use of ethylene glycol as a penetrating CPA; vitrification: use of ICSI, decreasing penetrating CPA concentrations in vitrification solutions, further increasing the cooling and warming rates by minimizing the sample volume and using open carriers
    e. Slow cooling: use of ICSI, addition of a sugar such as sucrose to cryopreservation solutions; vitrification: use of ICSI, full equilibration with final penetrating CPA concentrations in vitrification solutions, further increasing the cooling and warming rates by minimizing the sample volume and using open carriers

(continued)

3. Which one of the following accurately describes ovarian tissue cryopreservation outcomes?
    a. There have been fewer than 100 ovarian transplants with frozen-thawed ovarian tissue.
    b. Ovarian tissue vitrification is proven to be superior to slow freezing.
    c. Twin-twin transplantation is proven to be a cost-effective and successful alternative to oocyte donation for young women with premature ovarian failure.
    d. Pregnancy rates following ovarian tissue cryopreservation are comparable with oocyte cryopreservation rates.
    e. Studies have found an increase in birth defects associated with ovarian tissue cryopreservation.

4. Which one of the following is true regarding the IVF treatment for women with breast cancer?

    a. Ovarian stimulation concurrent with letrozole therapy is not associated with increased breast cancer recurrence in 5-year follow-up.
    b. In women with cancer, the most aggressive ovarian stimulation regimen is the best bet, since this is their only chance before chemotherapy.
    c. Patient's with Turner syndrome can never conceive and thus fertility preservation should never be discussed.
    d. Pregnancy after breast cancer has been shown to increase recurrence rates.
    e. Resumption of menstruation after breast cancer treatment assures that a woman will be fertile.

5. A 39-year-old female is diagnosed with breast cancer. She is interested in knowing what her chances of conceiving will be in the future. In counseling her, which one of the following is true?

    a. All types of chemotherapy have similar impact.
    b. Total dose of chemotherapy is not important.
    c. Her age at time of treatment is not significant.
    d. Age at the time she attempts to conceive is an important prognostic factor.
    e. Age at menarche is critical.

6. A 36-year-old female is diagnosed with endometrial cancer. She has a long history of irregular menses and has been told that she has polycystic ovary syndrome (PCOS). Fertility preservation options are discussed with her. Which one of the following is true about using in vitro maturation (IVM) for this patient?
    a. Increased risk of ovarian hyperstimulation syndrome (OHSS)
    b. Higher cost of medications
    c. Easier scheduling of oocyte retrieval
    d. More discomfort
    e. Shorter retrieval time

(continued)

7. Which one of the following is true regarding ovarian primordial follicle reserve?

    a. Current dogma is that primordial follicles are established at puberty.
    b. Maximum number is achieved at puberty.
    c. There is steady follicle loss throughout reproductive life.
    d. Presence of germ stem cells is controversial in humans.
    e. No study has suggested presence of germ stem cells in rodents.